Efficient synthesis of biologically active small molecules

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Efficient Synthesis of Biologically Active Small Molecules

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This research has been carried out under the supervision of

Dr Lorenzo Caggiano

For Mum and Dad.

“Ordinary riches can be stolen; real riches cannot. In your soul are infinitely precious things that cannot be taken from you.”

Oscar Wilde

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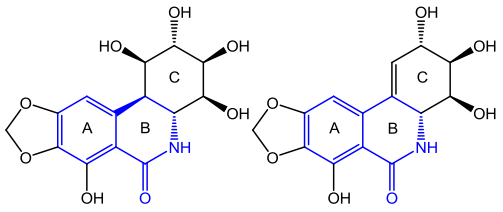
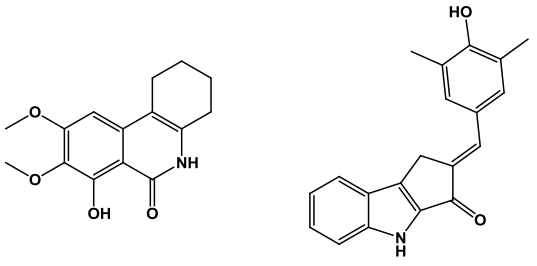
Abstract

Pancratistatin and narciclasine are natural products isolated from *Pancratium litorale*[1] and *Narcissus poeticus*[2] respectively. Pancratistatin and Narciclasine have been shown to possess potent antitumour activity[3] however they have never been widely exploited due to their limited availability from natural sources.[4] Pancratistatin and narciclasine both contain a dihydroisoquinolinone framework. The work described in this thesis explores synthetic routes relating to this dihydroisoquinolinone framework, as well as comparable tetrahydroisoquinolines. An initial proposed synthetic route involved the synthesis of the dihydroisoquinolinone framework *via* the corresponding indanone. Indanones have also been shown to possess potential antitumour activity.[5]



A range of lactam and indanone analogues were synthesised and a selection were tested for biological activity against cancer cell lines. The most biologically active lactam analogue synthesised was lactam **170.** Lactam **170** was synthesised *via* two steps from commercially available starting materials in an overall 51 % yield and was tested in the HT29 colon cancer cell line to give an IC_{50} value of 9 μM. Indanone **177** is an analogue of natural product indanocine and was synthesised *via* two steps in an overall 49 % yield. Analogue **177** was tested in the 60 cell line screen by the National Cancer Institute (NCI) to give a mean GI_{50} value of 1.29 μM and is currently under consideration for further testing. This thesis describes the synthesis and biological testing of the aforementioned compounds as well as an array of analogues.



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List of Abbreviations

aa-tRNA	Aminoacyl transfer ribonucleic acid
ACh	Acetylcholine
AcOH	Acetic acid
AgCl	Silver chloride
Ag_2CO_3	Silver carbonate
Ag_2O	Silver(I) oxide
$AlCl_3$	Aluminium chloride
aq.	Aqueous
Ar	Argon
ATP	Adenosine-5'-triphosphate
b.p.	Boiling point
^oC	Degrees Celsius
CH_2Cl_2	Dichloromethane
$CHCl_3$	Chloroform
CO_2	Carbon dioxide
DCE	Dichloroethane
DCP	Dicumyl peroxide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DISC	Death-inducing signalling complex
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
DNP	2,4-Dinitrophenylhydrazine
eEF1A	Elongation factor 1A
eq.	Equivalents
EtOAc	Ethyl acetate
Et_2O	Diethyl ether
EtOH	Ethanol
+ESI	Positive electrospray ionisation mode
$FeCl_3$	Iron(III) chloride
GI_{50}	Concentration required to inhibit growth by 50 %

H_2O	Water
H_2O_2	Hydrogen peroxide
H_3PO_4	Phosphoric acid
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
h	Hour
HRMS	High resolution mass spectrometry
Hz	Hertz
IR	Infrared
J	Coupling constant
K_2CO_3	Potassium carbonate
KOH	Potassium hydroxide
$LiOH.H_2O$	Lithium hydroxide
M	Molar
Me	Methyl
MeCN	Acetonitrile
MeI	Iodomethane
MeOH	Methanol
$MgSO_4$	Magnesium sulfate
mg	milligrams
mL	millilitres
mmol	millimoles
m.p.	Melting point
MS	Mass spectrometry
NaH	Sodium hydride
$NaHCO_3$	Sodium hydrogen carbonate
NaI	Sodium iodide
NaN_3	Sodium azide
NaOH	Sodium hydroxide
Na_2SO_4	Sodium sulfate
NCI	National Cancer Institute

$NH_4^+Cl^-$	Ammonium chloride
$nBuNI_4$	Tetrabutylammonium iodide
NMR	Nuclear magnetic resonance
N_2	Nitrogen
PE	Petroleum ether
$Pd(OAc)_2$	Palladium(II) acetate
PPA	Polyphosphoric acid
PPh_3	Triphenylphosphine
ppm	parts per million
PTZ	Pentylenetetrazol
room temp.	Room temperature
ROS	Reactive oxygen species
SAR	Structure activity relationship
sat.	Saturated
TBAB	Tetrabutylammonium bromide
TBAI	Tetrabutylammonium iodide
TBDMSCl	*tert*-butyldimethylsilyl chloride
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMEDA	Tetramethylethylenediamine
UV	Ultra violet

1. Chapter One - Introduction of Tetrahydroisoquinolines and Dihydroisoquinolinones

The aim of this project was to investigate the synthesis of both tetrahydroisoquinolines and dihydroisoquinolinones using established and new synthetic methods, as these are privileged motifs found in a variety of biologically active compounds (Figure 1).[6,7]

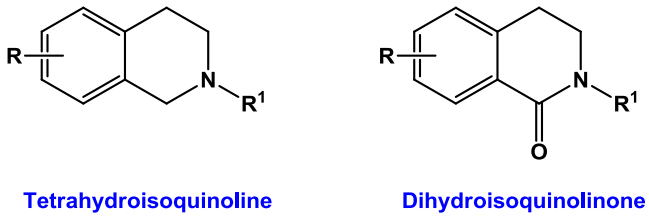


Figure 1: Structure of tetrahydroisoquinolines and dihydroisoquinolinones

1.1. 1,2,3,4-Tetrahydroisoquinolines

1.1.1. Biological Activity of 1,2,3,4-Tetrahydroisoquinolines

Many analogues of 1,2,3,4-tetrahydroisoquinolines have been found to possess antitumour activity,[8,9] have the potential to treat Alzheimer's disease[10] and are widely studied cytotoxic agents. Berberrubine, a protoberberine alkaloid exhibits antitumour activity in animal models (Figure 2).[11] It has also been previously reported that noscapine, a plant alkaloid, binds to tubulin and induces apoptosis selectively in tumour cells to display anticancer activity in ovarian and T-cell lymphoma cancers.[12] These examples demonstrate the utility of the 1,2,3,4-tetrahydroisoquinoline core and why these types of compounds are of great interest.

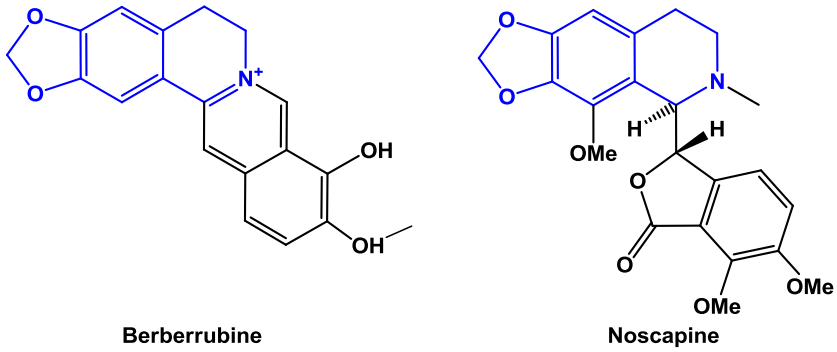
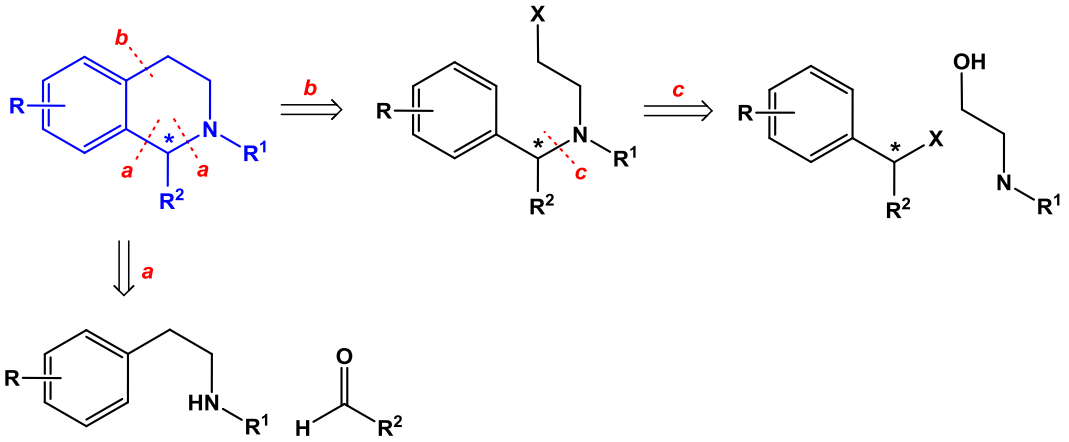


Figure 2: Berberrubine and noscapine are natural alkaloids that contain the 1,2,3,4-tetrahydroisoquinoline framework

1.1.2. Current Syntheses of 1,2,3,4-Tetrahydroisoquinolines

The existing syntheses of this framework include a Pictet-Spengler,[13-15] a Friedel-Crafts[16] or a Pomeranz-Fritsch[17] reaction.

The most popular synthetic approach to 1,2,3,4-tetrahydroisoquinolines has been the Pictet-Spengler reaction, which is shown by the disconnection at position ***a*** (Scheme 1).[15]

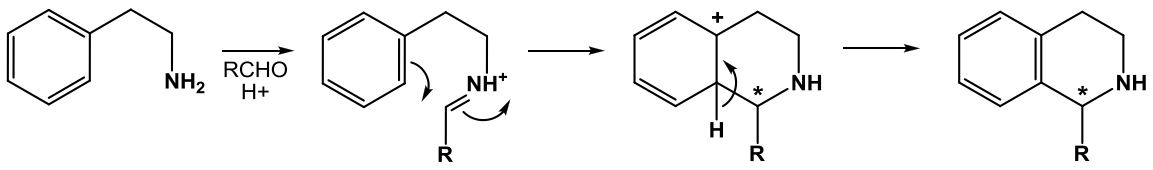


Scheme 1: Possible disconnection approaches to the synthesis of 1,2,3,4-tetrahydroisoquinolines

Although this reaction has been employed in many syntheses, it is limited by non-trivial synthesis of the arylethylamine starting material and lack of stereocontrol in the newly formed stereogenic centre at the C1 position. Following disconnection at position ***b*** affords more accessible starting materials from benzylation of a simple ethanolamine derivative. This disconnection involves either an intramolecular Friedel-Crafts cyclisation[16] or Pomeranz-Fritsch reaction but unfortunately these are typified by low yields and harsh reaction conditions.

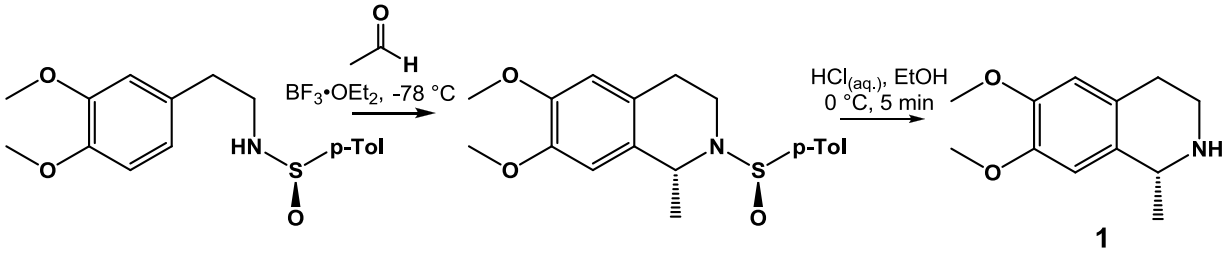
1.1.2.1 Pictet-Spengler reaction

The Pictet-Spengler reaction proceeds *via* the condensation of an arylethylamine with an aldehyde to give the desired 1,2,3,4-tetrahydroisoquinoline framework (Scheme 2).



Scheme 2: Mechanism of the Pictet-Spengler reaction

There is a lack of effective enantioselective synthetic routes to obtain an enantiomerically pure tetrahydroisoquinoline ring system. An effective method has previously been reported using a chiral auxiliary mediated Pictet-Spengler reaction (Scheme 3).[14] The first step in this synthesis involves the use of a chiral auxiliary which is removed using mild acid. Desired product **1** was synthesised in high yield (75 % over 2 steps) and with excellent stereoselectivity.

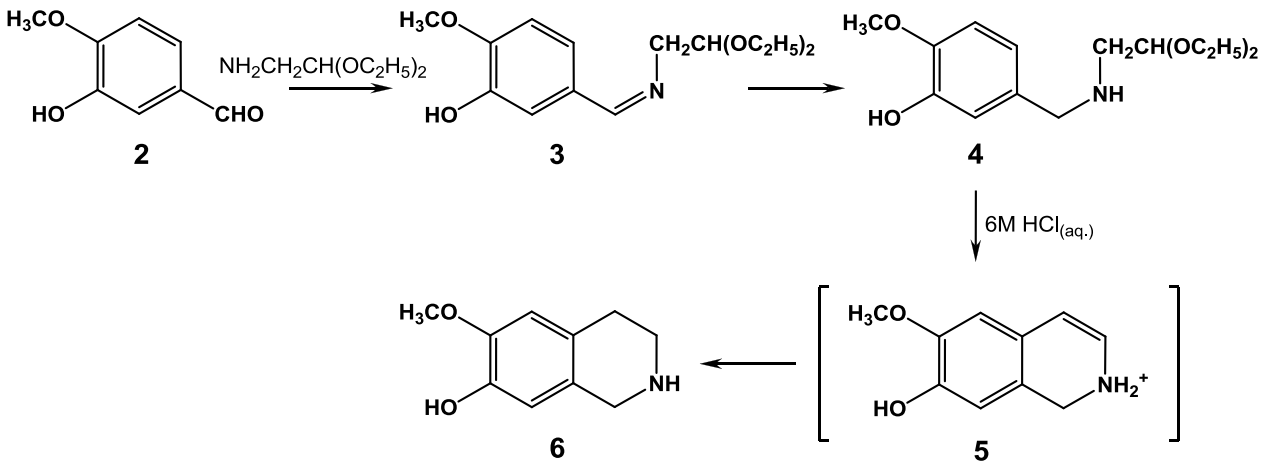


Scheme 3: Enantioselective Pictet-Spengler reaction using a chiral auxiliary

1.1.2.2 Pomeranz-Fritsch Reaction

1,2,3,4-Tetrahydroisoquinolines have been formed *via* a Pomeranz-Fritsch reaction following disconnection at position ***b*** (Scheme 1).

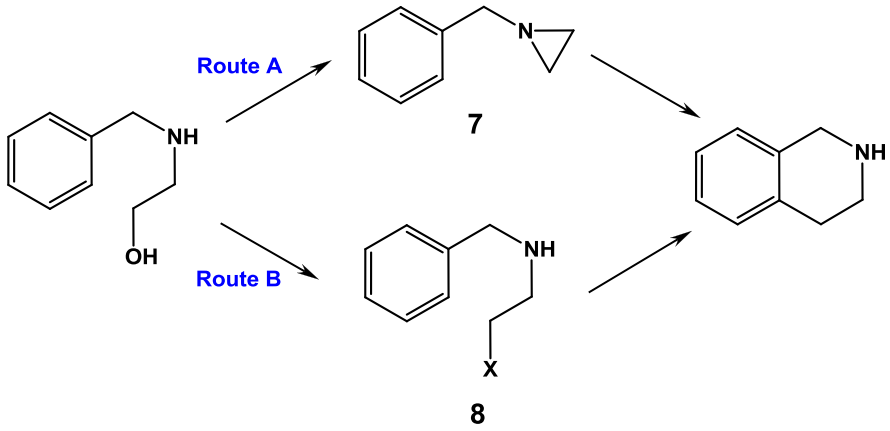
Bobbitt *et al.* reported the reaction of benzaldehyde derivative **2** and aminoacetaldehyde diethyl acetal in the presence of an acid to form imine **3**, which was subsequently catalytically reduced to the amine **4** (Scheme 4).[17] Amine **4** was then treated with 6M $HCl_{(aq.)}$ to form compound **5**. Hydrogenation of compound **5** afforded 1,2,3,4-tetrahydroisoquinoline **6** in good yield (80-90 %).



Scheme 4: Previously reported Pomeranz-Fritsch reaction to afford compound **6** in 80-90 % yield[17]

1.1.2.3 Intramolecular Friedel-Crafts Alkylation

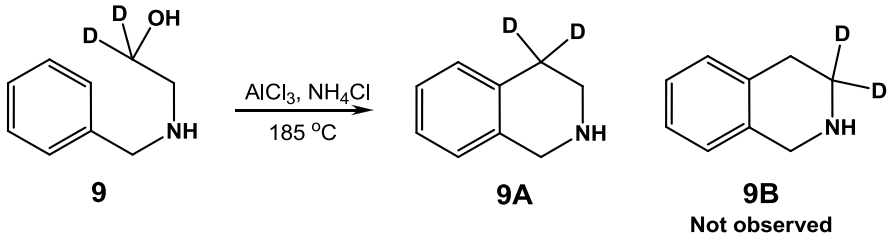
1,2,3,4-Tetrahydroisoquinolines have also been formed *via* a Friedel-Crafts alkylation reaction by disconnection at position ***b*** (Scheme 1), which requires an aromatic ring and an alkyl halide in the presence of a strong Lewis acid catalyst. Two mechanistic routes are possible for the cyclisation reaction, which are outlined in Scheme 5**.**



Scheme 5: Two possible mechanistic pathways to synthesise the tetrahydroisoquinoline core

The tetrahydroisoquinoline can be obtained directly from the amino alcohol *via* an aziridine **7** (Route A) or an amino-halide intermediate **8** (Route B).

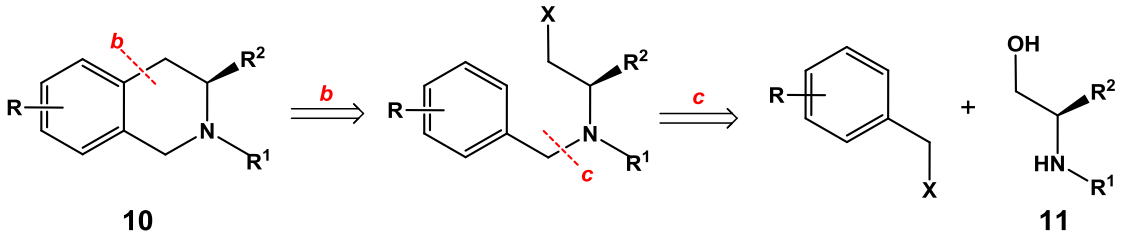
This pathway has been previously investigated using ammonium chloride with aluminium chloride at 185 ^{o}C and deuterium was used to label the ethanolamine **9** (Scheme 6).[18] If the aziridine were involved then both products **9A** and **9B** would be expected in approximately a 1:1 ratio. Only **9A** was isolated in 100 % yield indicating that the reaction proceeds exclusively *via* Route B and not by the aziridine intermediate.



Scheme 6: Deuterium labelled tetrahydroisoquinolines[18]

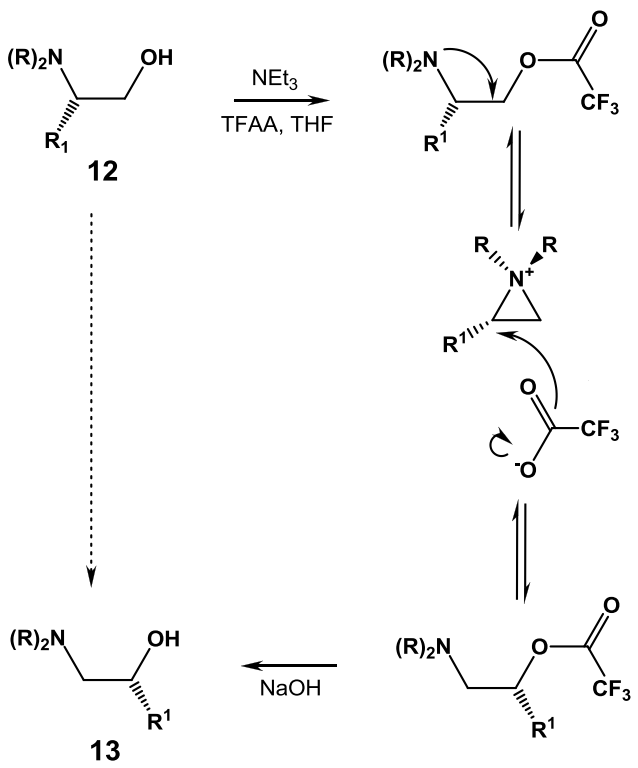
1.1.3. Proposed Synthetic Route *via* Alkylation

The proposed synthetic route to access the 1,2,3,4-tetrahydroisoquinoline core **10** exploits the disconnection approach at position ***b*** followed by position ***c*** (Scheme 1 and Scheme 7). Chirality can easily be introduced following this route (R^2) as the β-amino alcohol **11** can be synthesised in a one step reduction of a variety of naturally occurring, enantiomerically pure α-amino acids.



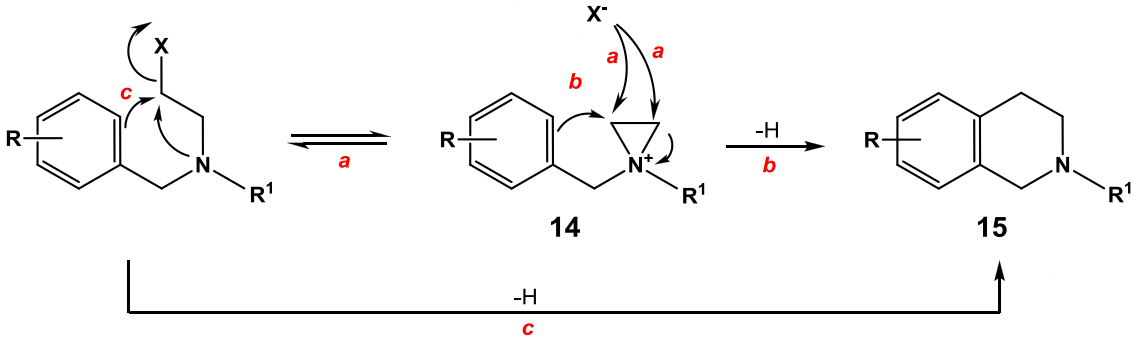
Scheme 7: Proposed disconnection approach to synthesise the 1,2,3,4-tetrahydroisoquinoline core *via* alkylation

β-amino alcohols **12** can be transformed into their α-substituted amino alcohols **13** selectively (Scheme 8).[19] Initial addition of trifluoroacetic anhydride (TFAA) activates the alcohol, followed by neighbouring group participation from the nucleophilic nitrogen to afford an aziridinium cation intermediate. Base hydrolysis then affords the rearranged amino alcohol in a regio-, stereo- and enantioselective method.



Scheme 8: The selective transformation of β-amino alcohols to their corresponding α-substituted amino alcohols *via* an aziridinium cation intermediate[19]

The proposed synthesis potentially involves a similar aziridinium ion intermediate **14** by neighbouring group participation (Scheme 9). It is hoped that the electron-rich aromatic ring will then cyclise by intramolecular attack to relieve the ring strain of aziridinium cation **14** to form the desired 1,2,3,4-tetrahydroisoquinoline core **15**.



Scheme 9: Possible mechanisms of the proposed synthesis

Direct attack would also be feasible in this disconnection approach (Route B, Scheme 5). Mendelson *et al.* used the same disconnection approach on a very similar substrate and discovered their reaction did not proceed *via* the aziridine intermediate (Scheme 6).[18]

1.2. Dihydroisoquinolinones

1.2.1. *Amaryllidaceae* Family of Plants

The *Amaryllidaceae* family of plants is found across the tropical and warm regions of the world and has proven to be a plentiful source for therapeutic agents. To date, up to 500 compounds have been isolated from this family of plants and several of these have been shown to possess a wide range of biological activities.[20] As a result, much attention has been paid to the isolation, identification and research into these natural products. From 2009-2011, nineteen new alkaloids have been isolated from the *Amaryllidaceae* family of plants.[21]

These plants are primarily used for their ornamental properties. However, when a cut daffodil is placed in a vase with other flowers, it can have a detrimental effect on the other plants and can shorten their vase life. Extracts from the bulb of the daffodil (*Narcissus*) were first exploited by the physician Hippokrates of Kos in Ancient Greece (B.C. 460-370), who recommended the use of a pessary prepared from *narcissus* oil to treat uterine tumours.[7]

1.2.2. Galanthamine

Galanthamine (Figure 3) is an alkaloid previously isolated from both the daffodil and snowdrop bulbs. This is the first example of a natural product isolated from the *Amaryllidaceae* family of plants which has been commercially approved (marketed in the UK as Reminyl by Shire Pharmaceuticals) and is used for the treatment of Alzheimer's disease.[22] Alzheimer's disease is a common form of dementia and previous research has revealed that the brains of sufferers show a diminished level of acetylcholine (ACh). Acetylcholine (ACh) is a neurotransmitter of the central nervous system, which increases awareness and learning.[23]

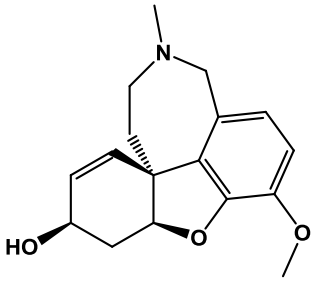


Figure 3: Galanthamine

Acetylcholinesterase is an enzyme responsible for the removal of ACh *in vivo*, therefore a common treatment for Alzheimer's disease is by the use of acetylcholinesterase inhibitors. Galanthamine is an example of an acetylcholinesterase inhibitor, which has been shown to slow the progression of the disease. This example highlights why there is great interest in this group of plants and the collection of compounds isolated from them.

1.2.3. Pancratistatin and Narciclasine

1.2.3.1 Isolation

Pancratistatin **16** and narciclasine **17** are two more examples of natural products isolated from the bulbs of the daffodil (Figure 4). Pancratistatin **16** and was first isolated from *Pancratium litorale* in 1984 by Pettit *et al.*[1] and narciclasine **17** from *Narcissus poeticus* in 1967,[2] which was fully characterised in 1968 by Piozzi *et al.*[24] Both contain the same basic dihydroisoquinolinone core structure.

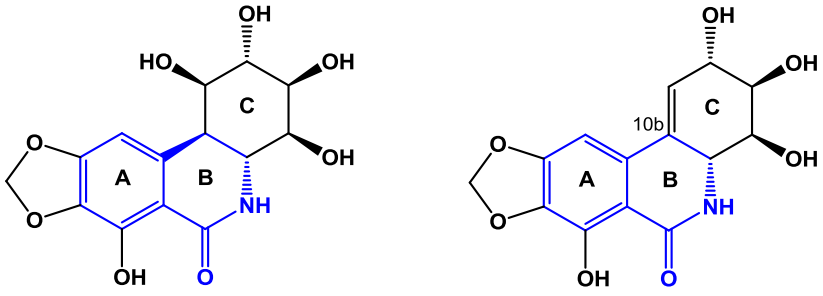


Figure 4: Pancratistatin **16** and narciclasine **17**

1.2.3.2 Biological Activity

These natural products are of great interest as they both possess potent antitumour activity that has previously been explored.[7] Pancratistatin **16** and narciclasine **17** have been tested against the National Cancer Institute (NCI) 60 cell line screen with mean GI_{50} values of 0.091 μM and 0.016 μM respectively.[7] In preliminary investigations they displayed antiviral activity in mice with the Japanese encephalitis virus but only at near toxic concentrations and with low selectivity.[25]

Little is known about the precise mode of action of these compounds. There is increasing interest in the biological research of pancratistatin **16** and narciclasine **17** in order to establish their exact anticancer mode of action and some preliminary studies on the natural products have previously been reported and are now described.

In 1975, Carrasco and co-workers reported that narciclasine **17** inhibits eukaryotic protein synthesis by interfering with peptidyl transferase by specifically targeting the 60S ribosome subunit of the A site (Figure 5).[26]

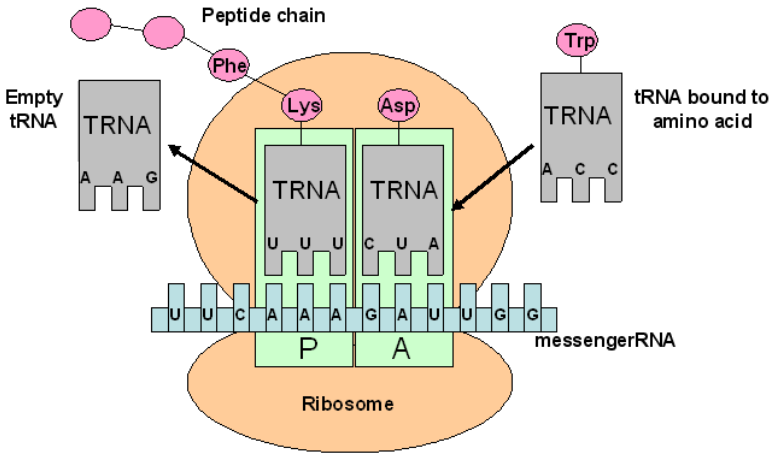


Figure 5: Illustration representing translation during peptide synthesis[adapted from27]

eEF1A is a protein which delivers aa-tRNA to the empty A site of the ribosome during peptide synthesis. It has recently been reported that narciclasine **17** binds to the eEF1A, therefore preventing the delivery of the aa-tRNA to the A site and subsequently inhibiting peptide synthesis.[28]

Melanoma is an extremely aggressive form of skin cancer and is resistant to many forms of anticancer therapies.[29] One third of early-stage melanoma patients will suffer from metastases and patients with metastatic melanomas unfortunately have a survival period of up to 6 - 8 months.[28] Narciclasine has been shown to display IC_{50} values between 30-100 nM in melanoma cell lines, irrespective of their levels of resistance.

Cell apoptosis can be triggered through two different pathways; the first is the death receptor pathway, also known as the extrinsic pathway, which is initiated through the stimulation of transmembrane death receptors on the cell membrane. The second is *via* the mitochondrial pathway, also known as the intrinsic pathway and is directly initiated at the mitochondria within the cell.[30]

In 2007, Dumont *et al.* reported that narciclasine **17** induces apoptosis selectively in a range of cancerous cells compared to normal human lung fibroblasts.[31]

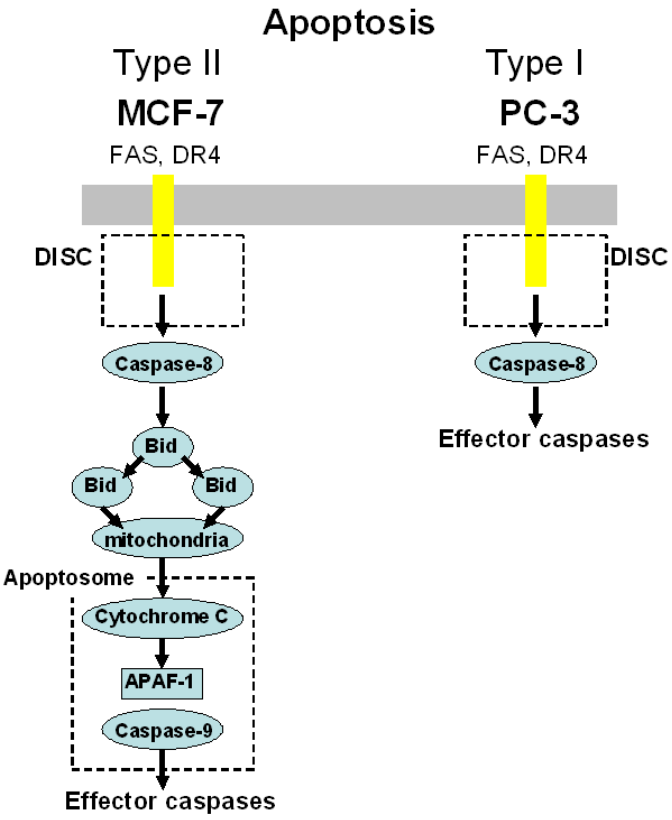


Figure 6: Summary of the induced apoptosis pathways by narciclasine **17**[adapted from 31]

In this same report, when PC-3 prostate and MCF-7 breast cancer cell lines were treated with narciclasine; both death receptors FAS and DR4 were stimulated, leading to the activation of the death-inducing signalling complex (DISC) (Figure 6).

PC-3 prostate cancer cells behave like type 1 cells on treatment with narciclasine **17**, where the required concentration of caspase-8 is sufficient and triggers the caspase cascade, resulting in cell death. MCF-7 breast cancer cells behave like type 2 cells where these cells lack the required concentration of caspase-8 and amplify the apoptotic signal by triggering the mitochondrial pathway, resulting in the release of cytochrome c, consequently causing cell death.

Current cancer treatments cause DNA damage in both healthy and cancerous cells. For future treatments to be selective, the differences between normal and cancerous cells need to be exploited. Previous studies have shown a difference between the mitochondria of cancerous cells and those of non-cancerous cells, therefore making the mitochondria an attractive target for potentially selective anticancer treatment.[32]

In 2005, McLachlan *et al.* reported that pancratistatin **16** selectively induces apoptosis at 1 μM concentrations within 24 hours in cancerous cells but does not appear to have an effect on healthy cells at the same concentration, even after 96 hours of treatment.[33] This same study also revealed that the mitochondria appear to be the sites of action of pancratistatin **16**. The amount of reactive oxygen species (ROS), which are linked to mitochondrial disfunction, have been shown to increase when cancer cells are treated with pancratistatin **16**, while normal cells do not show this increase.

The role of the mitochondria is to produce energy for cells in the form of ATP. If there is a decrease in ATP, then this also indicates mitochondrial disfunction. McLachlan *et al.* revealed that the cellular concentrations of ATP in cancerous cells decrease during treatment with pancratistatin **16**.[33] Normal cells do not show any change in the concentration of ATP on treatment with pancratistatin **16**.

The tumour-suppressor protein p53 activates the pro-apoptotic proteins in the cytoplasm, BAX and BID, in the intrinisic apoptotic pathway. Current cancer treatments require p53 to work effectively; however, cancers mutate and become resistant to these treatments. More recently, Griffin *et al.* reported that pancratistatin **16** induces apoptosis in both p53-negative (HT-29) and p53-wild type (HCT116) colon cancer cell lines, without showing toxicity to normal colon cells (CCD-18Co).[34]

1.2.3.3 Structure-Activity Relationship of Pancratistatin

Previous research on pancratistatin **16** has identified several structural features that are required for its anticancer activity (Figure 7).[7] The A-ring must contain at least three oxygen substituents and the removal of the free phenol at the C7 position (7-deoxypancratistatin) on the A-ring has been shown to decreases the activity of pancratistatin 10-fold.[35] The fused B/C ring junction must be *trans* in order to retain the activity, which was demonstrated in 2002 by Hudlicky *et al.* where the *cis* epimer of 7-deoxypancratistatin was inactive against six cancer cell lines.[36] The C-ring must contain at least three hydroxy groups in order to retain cytotoxic activity,[37,38] although the required relative and absolute configuration of these hydroxy groups is not yet known.

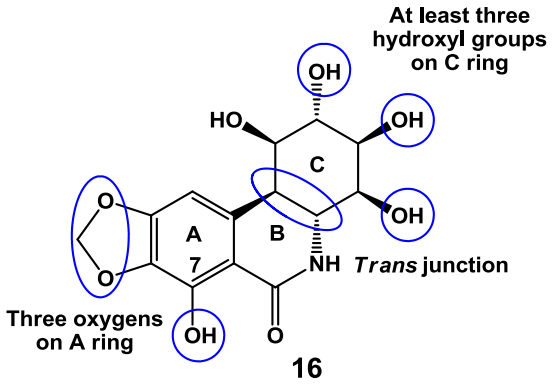


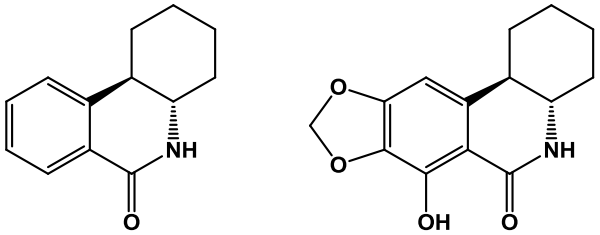
Figure 7: Structural elements required for the anticancer activity of pancratistatin **16**

To date, no alterations to the lactam present in the B-ring have been shown to improve activity compared to the natural product. The corresponding lactone of 7-deoxypancratistatin tested in L1210, a mouse lymphocytic leukaemia cell line, showed no significant biological activity.[39]

1.2.3.4 Current Syntheses of Pancratistatin

The first total synthesis of (+/−)-pancratistatin was reported in 1989 by Danishefsky *et al.*[40] The synthesis took a total of 26 steps to complete and gave an overall 0.13 % yield.

Heathcock *et al.* in 1992 published work on the successful synthesis of two analogues of pancratistatin, **18** and **19**, containing the dihydroisoquinolinone lactam core (Figure 8).[41] Their aim was to develop an efficient synthesis of these analogues and achieve sufficient quantities to enable the subsequent application of these compounds in clinical trials.



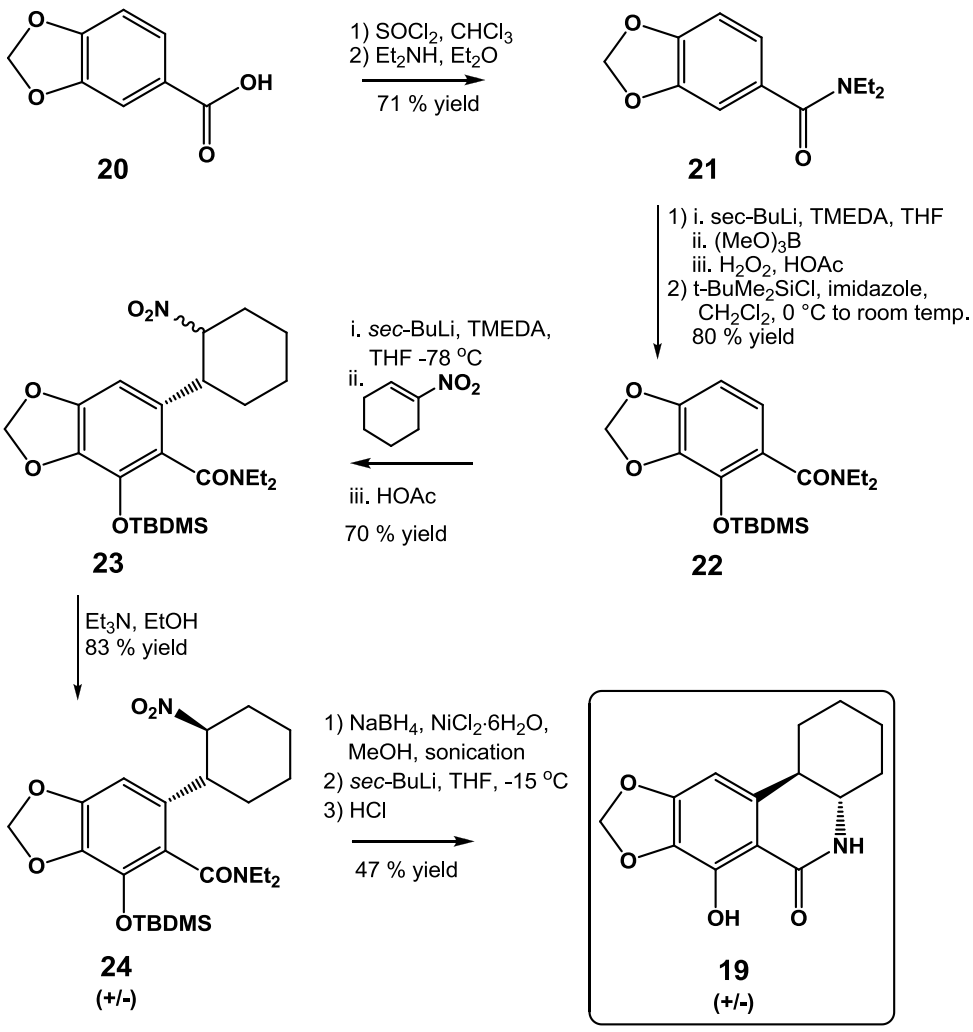
18 (+/-) **19** (+/-)

Figure 8: Pancratistatin skeleton models **18** and **19**[41]

The synthesis of model compound **18** was achieved in only 4 steps and the synthesis of analogue **19** was synthesised in only 9 steps, both through a similar synthetic strategy.

Piperonylic acid **20** was transformed into the acid chloride, which was then treated with diethylamine in diethylether to give the corresponding amide **21** in 71 % yield (Scheme 10). Metalation at the *ortho* position,[42] followed by the addition of trimethyl borate, afforded the corresponding boronate, which was oxidised with 30 % aqueous H_2O_2 and acetic acid. The resulting crude phenol was protected as the *tert*-butyldimethylsilyl ether **22** in 80 % yield.

The tertiary amide directs *ortho* lithiation for a second time on intermediate **22** followed by the addition of 1-nitrocyclohexene in THF to afford a mixture of *cis* and *trans* 1,4-addition product **23** in 70 % yield. This underwent epimerisation on treatment with Et_3N in ethanol to yield pure *trans* isomer **24**. The nitro group was then reduced to the amine which, without purification, underwent cyclisation to obtain the desired model compound **19** in 47 % yield.



Scheme 10: Synthesis of tricyclic skeleton **19**[41]

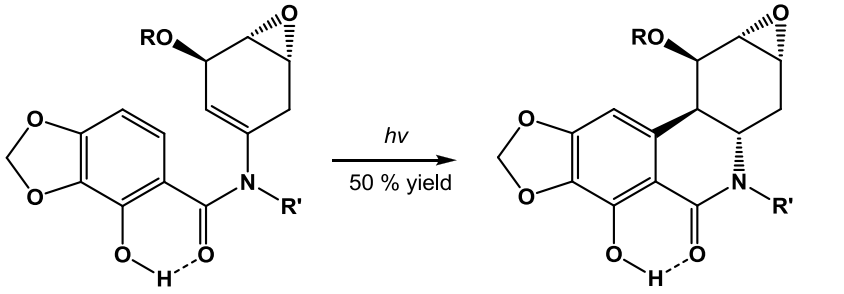
In 1995, Tian *et al.* published the first enantioselective synthesis of (+)-pancratistatin *via* a 14-step synthesis with an overall 2 % yield.[43] Later that same year, another asymmetric synthesis of (+)-pancratistatin was published by Trost and Pulley, which took 15 steps and gave a greater overall yield of 11 %.[44]

Over the following years, many groups published enantioselective syntheses of (+)-pancratistatin, which entail a large number of steps and low overall yields (Table 1).

Research group	Year	Number of steps	Overall yield (%)
Hudlicky[43]	1995	14	2.0
Trost[44]	1995	15	11.0
Doyle[45]	1997	24	1.0
Magnus[46]	1998	19	1.2
Rigby[47]	2000	22	0.4
Pettit[48]	2001	10 steps from narciclasine	3.6
Kim[49]	2002	16	4.0
Ko[50]	2004	17	5.8
Li[51]	2006	12	9.0

Table 1: Previously reported total syntheses of (+)-pancratistatin

The main transformation in the 22 step total synthesis reported by Rigby *et al.* in 2000 involves a hydrogen bond-controlled aryl enamide photocyclisation to afford the desired *trans* B/C ring junction intermediate **25** (Scheme 11).[47]

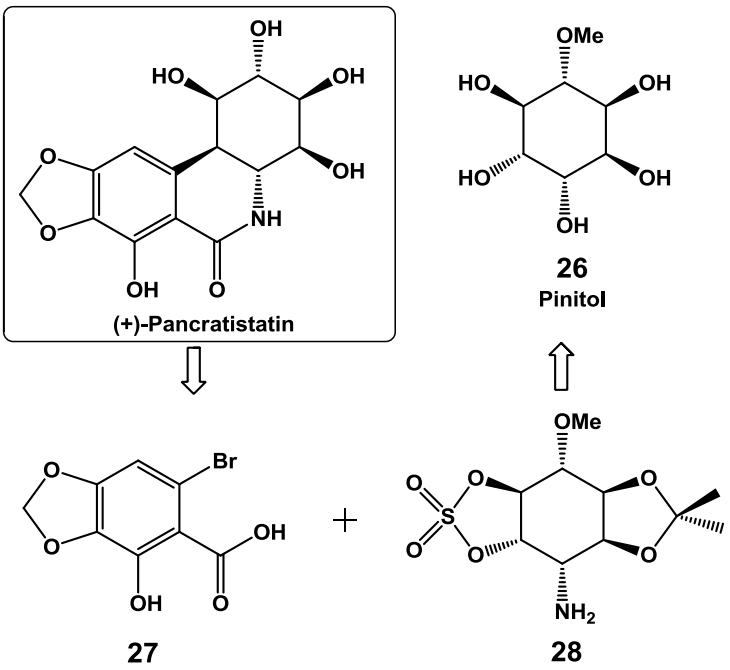


25

Scheme 11: Photocyclisation step in the reported total synthesis of pancratistatin by Rigby *et al.*[47]

The synthetic conversion of (+)-pancratistatin from (+)-narciclasine by Pettit *et al.*, published in 2001,[48] provided the opportunity to study the structure activity relationship of pancratistatin further. Narciclasine is more easily isolated and so is available in more practical quantities than is pancratistatin and, therefore, was an attractive precursor to study.

In 2006, Li *et al.* reported the synthesis of (+)-pancratistatin *via* 12 steps from pinitol **26** (Scheme 12).[51] Their synthesis employs the use of two coupling fragments, bromide intermediate **27** and compound **28**, which was synthesised from pinitol **26**.



Scheme 12: Retro-synthetic approach of (+)-pancratistatin using pinitol **26** as starting material[51]

In 2004, Hudlicky *et al.* speculated that a factor which may explain the activity of pancratistatin **16** could be the hydrogen-bonding donor-accepting pairing of the β-ketoamide framework.[52] They consequently explored the replacement of this core with an indole moiety to synthesise analogues which would hopefully mimic the anticancer activity of pancratistatin **16** (Figure 9). Molecular models of pancratistatin **16** and analogue **29** showed similar spatial similarities and a variety of indole analogues were synthesised and tested against 4 cancer cell lines.

These cancer cell lines include P388 (lymphocytic leukaemia), BXPC-3 (pancreatic adenocarcinoma), MCF-7 (breast adenocarcinoma) and KM20L2 (colon adenocarcinoma). Pancratistatin **16** displayed promising anticancer activity in the cell lines with IC_{50} values ranging from 0.062 μM - 0.138 μM. In comparison, compound **29** unfortunately displayed only slight activity in P388 lymphocytic leukaemia cell and no activity in the other three cell lines.[52]

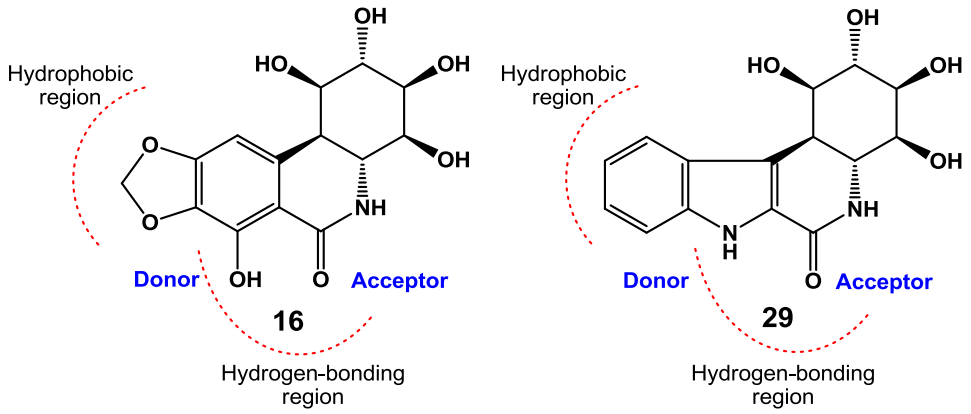


Figure 9: Synthesis of indole analogue **29** which shows similar spatial similarities to pancratistatin[52]

More recently, de la Sovera *et al.* envisioned that utilising a triazole **30** to replace the aromatic ring in pancratistatin **16** could be an interesting replacement (Figure 10).[53] The group synthesised four compounds containing the triazole core and are currently looking into the biological results of these new analogues.

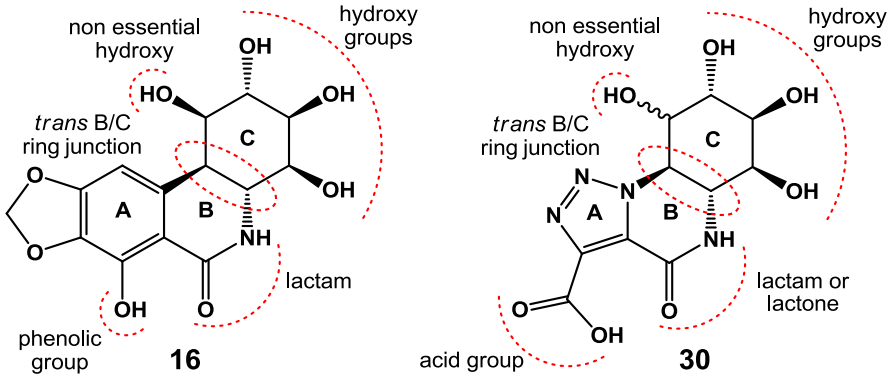


Figure 10: Analogue of pancratistatin which contains a triazole core **30**[53]

Vshyvenko *et al.* have synthesised two C-1 analogues of pancratistatin in 17 steps from bromobenzene (Figure 11).[54] Initial biological results show promising anticancer activities in four cancer cell lines, with analogues **31** and **32** showing IC_{50} values of 0.06 μM and 0.09 μM, respectively, in prostate cancer cell line HTB-81. Ma *et al.* also discovered that analogue **31** selectively induces apoptosis in pancreatic carcinoma cells.[55]

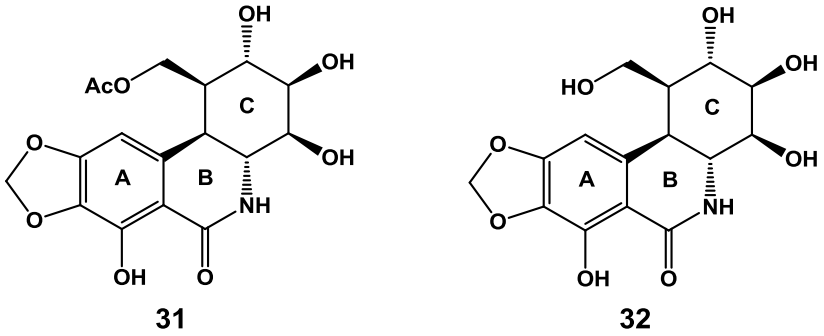


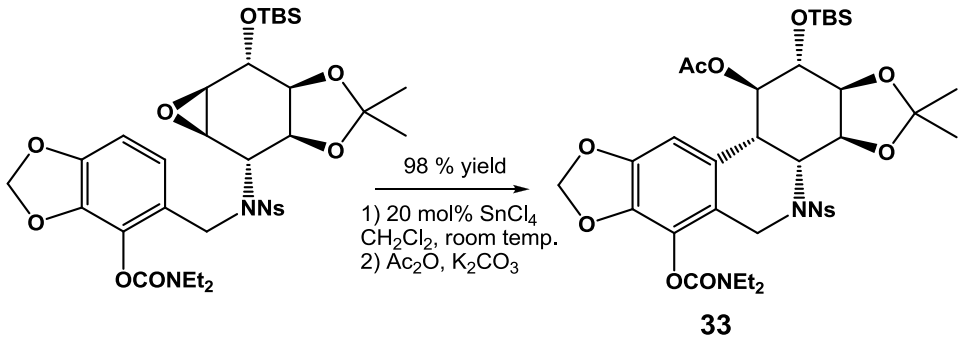
Figure 11: Novel C-1 analogues of pancratistatin, **31** and **32**[54]

1.2.3.5 Current Syntheses of Narciclasine

The first synthesis of (+)-narciclasine was reported in 1997 by Rigby and Matteo, which was accomplished in 23 steps with an overall 0.2 % yield.[56]

Two following enantioselective syntheses of (+)-narciclasine were reported in 1999. Gonzalez *et al.* achieved a 0.6 % overall yield in a total of 12 steps[57] and Keck *et al.* exploited the use of vinyl radicals and oxime ethers in their 14-step synthesis, with an overall yield of 11 %.[58]

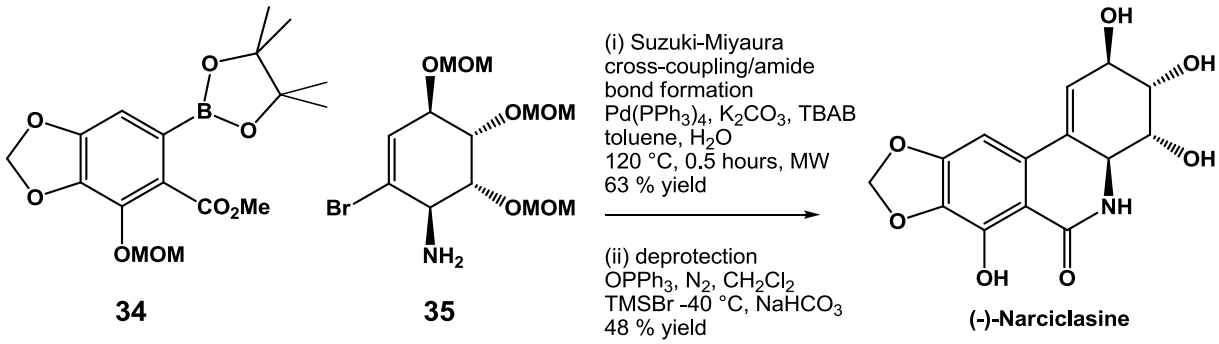
A more recent example by Yan *et al.* reported a 9-step synthesis with an impressive total yield of 17 %.[59] This short synthesis involved the use of stereocontrolled epoxidation followed by a $SnCl_4$ intramolecular arylation to yield the *cis* intermediate **33** (Scheme 13).



Scheme 13: $SnCl_4$-catalysed intramolecular cyclisation to afford compound **33**[59]

Most recently, Matveenko *et al.* reported a convergent synthesis of (−)-narciclasine in an overall yield of 7 %.[60] Intermediate **34** was synthesised in 10 steps and intermediate **35** in 9

steps (Scheme 14). It is not known whether the Suzuki-Miyaura cross-coupling reaction occurs before or after the amide bond forming reaction but, when the two intermediates were subjected to a Suzuki-Miyaura cross-coupling reaction under microwave conditions, followed by deprotection, the desired (−)-narciclasine was isolated.



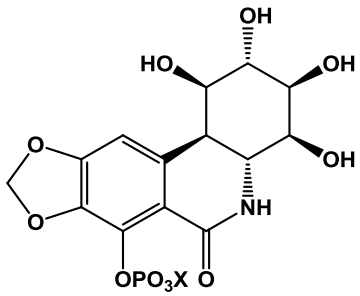
Scheme 14: Synthesis of (−)-narciclasine using intermediates **34** and **35**[60]

1.2.3.6 Drug Development of Pancratistatin and Narciclasine

Although the biological activity of pancratistatin and narciclasine is known, they have never been widely exploited due to their limited availability from natural sources; isolation can also be very costly. Petitt *et al.* isolated 6.5 g of pancratistatin from 45 kg of daffodil bulbs[3] and, since the first isolation of narciclasine in 1967,[2] 32 species of bulb have been studied for narciclasine content, which only typically yields 100-120 mg/kg.[61]

In addition the clinical development of pancratistatin has been hampered due to its poor aqueous solubility (<53 μg mL^{-1}),[62] therefore an effort has been made to develop prodrugs. In 2000, Pettit *et al.* synthesised a large range of phosphate prodrugs and tested both their solubility and activity in a range of cancer cell lines (Figure 12).[62]

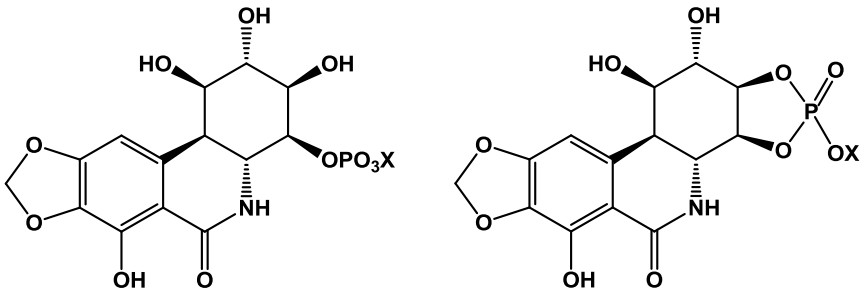
The use of the phosphate prodrugs considerably improved their aqueous solubility and these were found to hold similar activities against the murine P388 lymphocytic leukaemia cell line (IC_{50} values ranging from 0.033 μM to 0.086 μM) when compared to the IC_{50} value of 0.065 μM for the parent compound.



X = 2 Na^+, 2 Li^+, 2 K^+, 2 Rb^+, 2 Cs^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}

Figure 12: Range of phosphate prodrugs tested for solubility and biological activity[62]

Pettit *et al.* then went on to synthesise a second generation of phosphate prodrugs using the other hydroxy groups of pancratistatin (Figure 13).[63] Many of the phosphate prodrugs exhibit good activity against the murine P388 lymphocytic leukaemia cell line, with IC_{50} values ranging from 0.039 - 0.422 μM.



X = 2 Li^+, 2 K^+, Mg^{2+}, Ca^{2+}, Zn^{2+} X = Na^+, Li^+, K^+

Figure 13: A second generation of phosphate prodrugs tested for solubility and their biological activity against the murine P388 lymphocytic leukaemia cell line[63]

Due to the promising biological activities of pancratistatin and narciclasine and their limited availability from natural sources, there is a great interest in finding a short and efficient synthesis of these compounds. The natural products themselves are not good medicinal candidates due to their poor aqueous solubility. Previous strategies have involved attempting the total synthesis of either pancratistatin **16** or narciclasine **17** and subsequently working around disadvantages of the natural products by developing prodrugs for example.

1.3. Project Aims and Objectives

The primary aim of this project was to develop new syntheses for a range of tetrahydroisoquinoline and dihydroisoquinolinone analogues and to explore their biological activities.

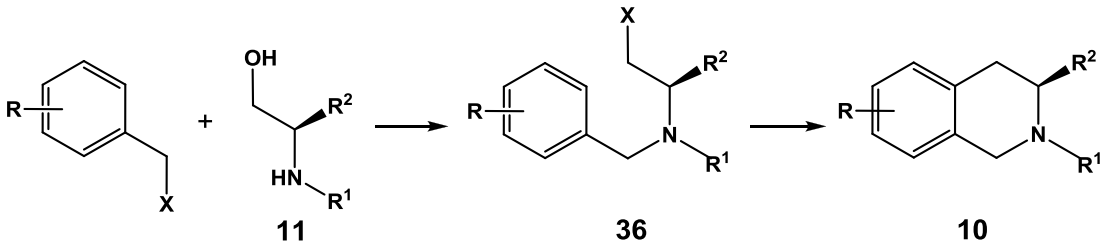
The dihydroisoquinolinone framework is present in many natural products, including pancratistatin **16** and narciclasine **17**. The aim was to access simple dihydroisoquinolinone analogues *via* straightforward chemistry, using a short and efficient synthesis from commercially available starting materials. The strategy was to first synthesise a simple A/B framework of the natural products pancratistatin **16** and narciclasine **17**. Once the A/B framework synthesis was established the intention was to develop this further and apply this synthetic strategy to access the A/B/C framework of pancratistatin **16** and narciclasine **17**.

Once the A/B/C framework method has been established alternative starting materials can be explored to access a range of novel non-natural analogues. This approach, if successful, gives access to a potentially large library of biologically active compounds. The aim was then to test these compounds for biological activity and any promising candidates could potentially be synthesised on a large enough scale for clinical application.

2. Chapter Two - 1,2,3,4-Tetrahydroisoquinolines

2.1. Proposed Synthesis *via* Friedel-Crafts Alkylation

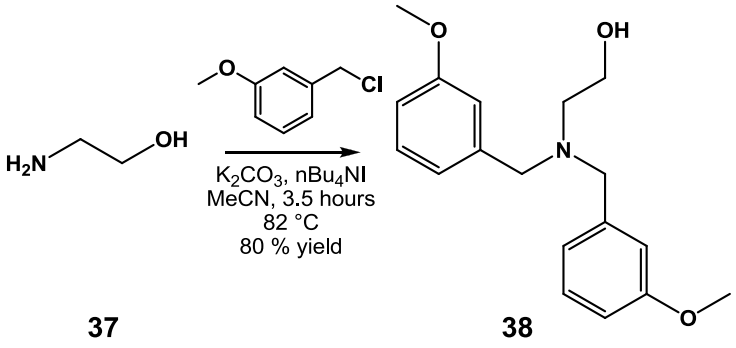
1,2,3,4-Tetrahydroisoquinolines were initially investigated. The proposed synthetic route *via* alkylation has been attempted with the initial synthesis of model substrate intermediates **36** (Scheme 15).



Scheme 15: Proposed synthesis of 1,2,3,4-tetrahydroisoquinolines *via* alkylation

2.1.1. Synthesis of Model Substrates

Model substrates were synthesised to attempt the intramolecular cyclisation by a tethered aromatic ring system. Bis-alkylation of commercially available ethanolamine **37** with 3-methoxybenzyl chloride, potassium carbonate and *n*Bu_4NI at reflux in MeCN for 3.5 hours afforded amino alcohol **38** in 80 % yield (Scheme 16). It is possible that the other 20 % has also alkylated at the alcohol position or alkylated at the nitrogen to afford the ammonium salt; however, no other products were isolated.

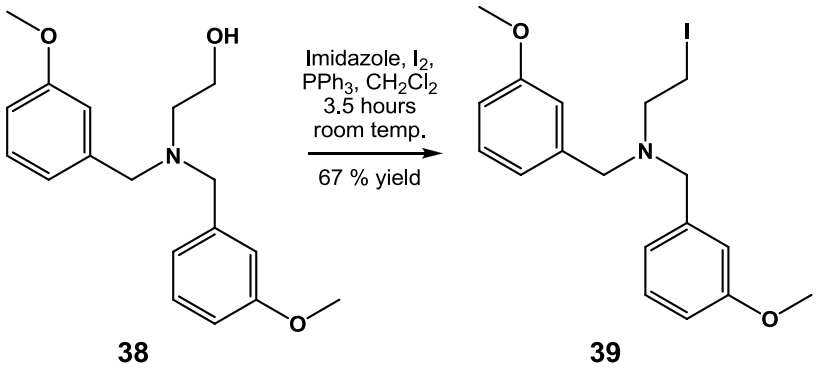


Scheme 16: Bis-alkylation of ethanolamine **37** to yield desired amino alcohol **38**

nBu_4NI is used to catalyse the reaction. Iodide is a good nucleophile so easily displaces the chloride of the 3-methoxybenzyl chloride resulting in 3-methoxybenzyl iodide. This more

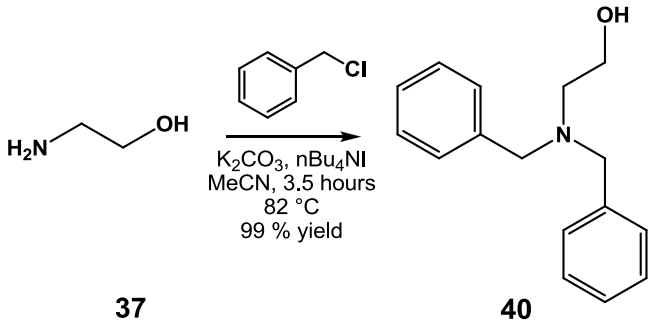
potent alkylating agent reacts to form the desired amino alcohol **38**. 3-Methoxybenzyl chloride was used as it is electron-rich, which may also promote the cyclisation reaction. The proposed novel synthesis also utilises an iodide as an intermediate to encourage the cyclisation reaction.

To form iodide intermediate **39**, amino alcohol **38**, I_2, PPh_3 and imidazole were stirred vigorously at room temperature for 3.5 hours to afford desired iodide **39** in 67 % yield (Scheme 17).



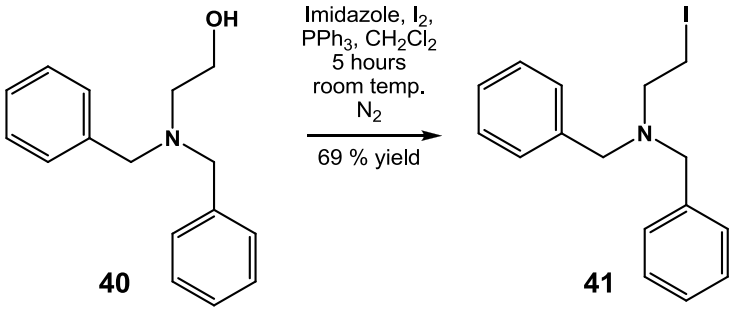
Scheme 17: Formation of iodide intermediate **39** from amino alcohol **38**

Non-substituted benzyl alcohol was also synthesised as an intermediate to explore whether an electron-rich substituent is required for cyclisation. Amino alcohol **40** was synthesised in 99 % yield (Scheme 18).



Scheme 18: Synthesis of amino alcohol **40** from ethanol amine **37**

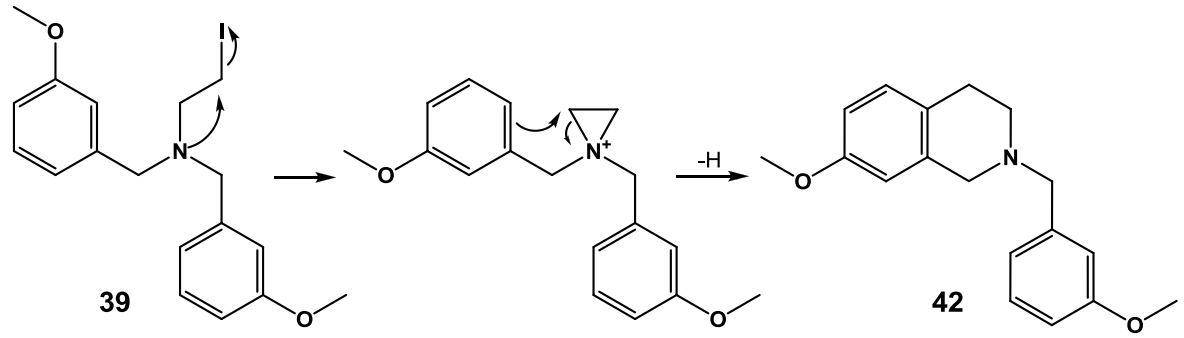
Iodide intermediate **41** was then synthesised from amino alcohol **40** with I_2, PPh_3 and imidazole at room temperature for 5 hours in 69 % yield (Scheme 19).



Scheme 19: Formation of iodide intermediate **41** from amino alcohol **40**

2.1.2. Attempted Cyclisations

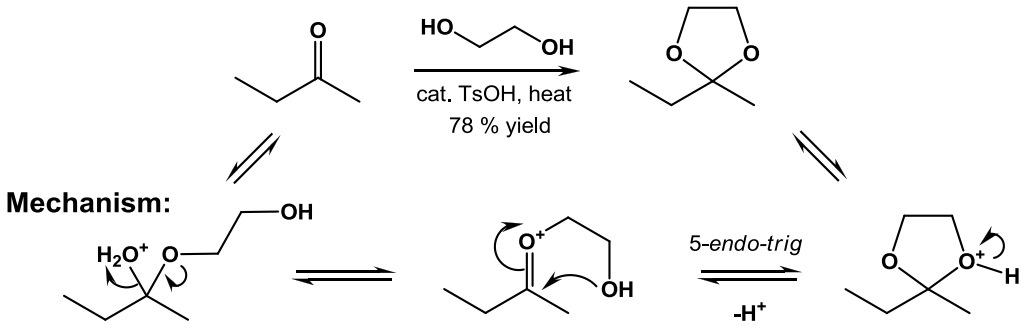
Iodide has been used as the leaving group rather than trifluoroacetic anhydride (TFAA) as reported by Metro *et al*. (Scheme 8).[19] The pKa of HI is -10 compared to ~0 for CF_3CO_2H indicating that iodide is a much better leaving group. Neighbouring group participation by the nitrogen under the reaction conditions could displace the iodide generating an aziridinium ion intermediate. The tethered ring system could then potentially undergo nucleophilic attack on the highly strained 3-membered ring to afford 1,2,3,4-tetrahydroisoquinoline **42** (Scheme 20).



Scheme 20: Cyclisation *via* aziridinium intermediate to afford 1,2,3,4-tetrahydroisoquinoline **42**

This ring-closure is described as a 5-*endo-tet* cyclisation which, according to Baldwin's Guidelines, are kinetically disfavoured. Baldwin's guidelines are a set of empirically derived Guidelines as to which cyclisations are favoured and which are not. The mechanistic rationale for these guidelines is based on the 3-D geometry of the orbitals which have to interact. 5-*Endo-tet* cyclisations are not favoured by Baldwin's Guidelines, although there are exceptions, including a well-known 5-*endo-trig* anomaly of the formation of a cyclic acetal

from a carbonyl compound and ethylene glycol (Scheme 21). This example is electrophilically led, which is similar to the proposed reaction shown in Scheme 20.

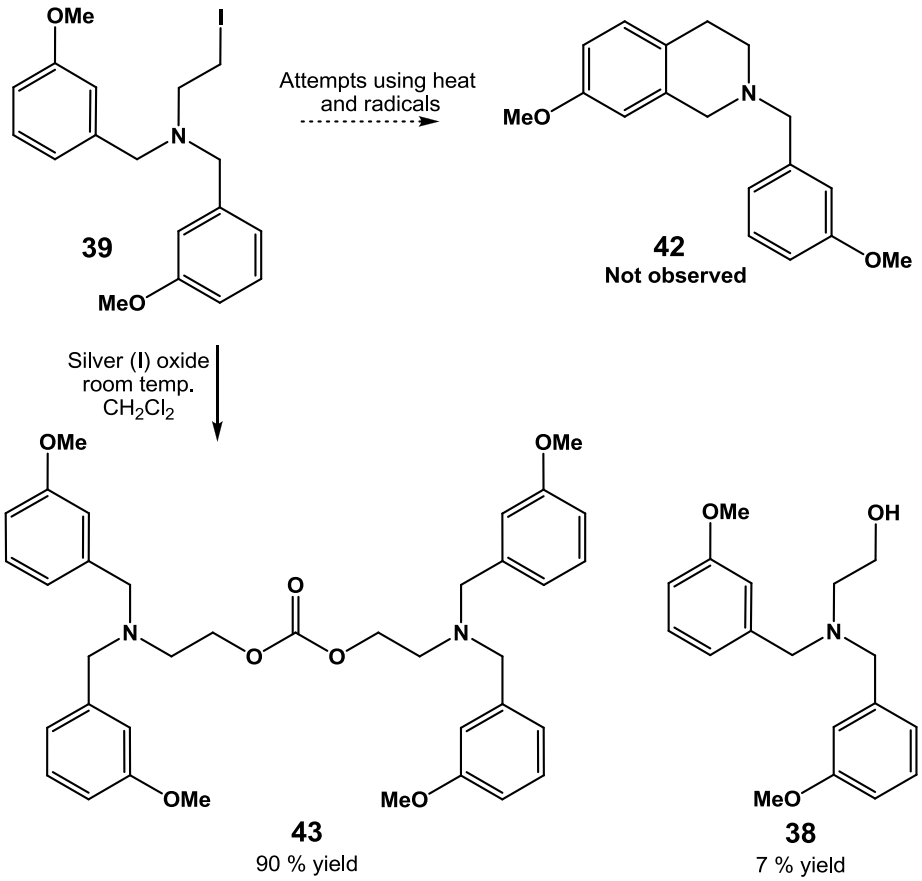


Scheme 21: 5-*Endo-trig* anomaly

Attempts to generate tetrahydroisoquinoline **42** from iodide **39** were made using heat and radicals (Scheme 22). Gentle heating did not encourage the formation of the desired product **42** and then forcing the reaction by heating at 100 ^{o}C for 72 hours caused the substrate to decompose without formation of the desired cyclised product **42**. The use of dicumyl peroxide (DCP), a 'tin-free' radical, was also attempted.[64] DCP decomposes to form a methyl radical which is an extremely reactive species. This should then react with the carbon-iodide bond to subsequently promote cyclisation of the aromatic system. Unfortunately, the desired cyclic product **42** was not observed in any of these reactions.

As previously mentioned, iodide is a powerful nucleophile and therefore there is competition between the iodide and the tethered aromatic ring to attack the aziridinium ion. When the iodide attacks the aziridinium ion the starting material is reformed. Silver oxide was added to sequester the iodide in an attempt to prevent it from attacking the aziridinium ion. This forces the equilibrium to form the aziridinium ion and the subsequent attack from the tethered aromatic nucleophile to form the desired product.

To test this hypothesis, the reaction of alkyl iodide intermediate **39** and Ag_2O in CH_2Cl_2 at room temperature, open to the air was attempted. The cyclic compound **42** was not obtained; instead the unexpected symmetrical carbonate product **43** was isolated in 90 % yield along with the corresponding alcohol **38** in 7 % yield (Scheme 22).



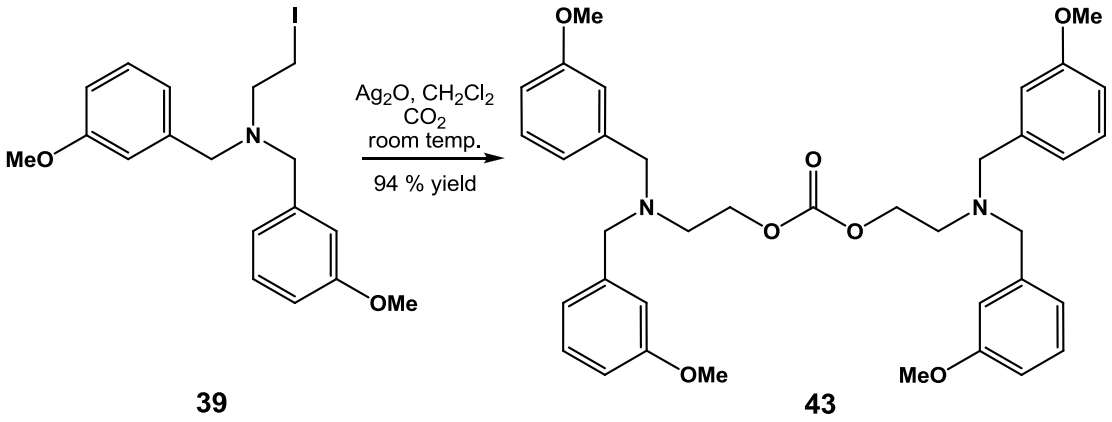
Scheme 22: Isolation of unexpected carbonate **43** in 90 % yield

The presence of the carbonyl group was determined by IR (1744 cm^{-1}) and ^{13}C NMR (δ 155.09 ppm), although initially it was unclear how this carbonyl group had been trapped within the molecule. Unfortunately, this reaction was not reproducible and only starting iodide **39**, along with its corresponding alcohol **38**, were isolated.

Silver oxide is a sorbent for CO_2 which is used to purify air within space suits worn by astronauts.[65] Carbon dioxide is absorbed by silver oxide to produce silver carbonate:

$$Ag_2O\ (s) + CO_2\ (g) \rightarrow Ag_2CO_3\ (s)$$

The same reaction was then repeated but this time under an atmosphere of CO_2 and the symmetrical carbonate **43** was isolated in a reproducible 94 % yield (Scheme 23).

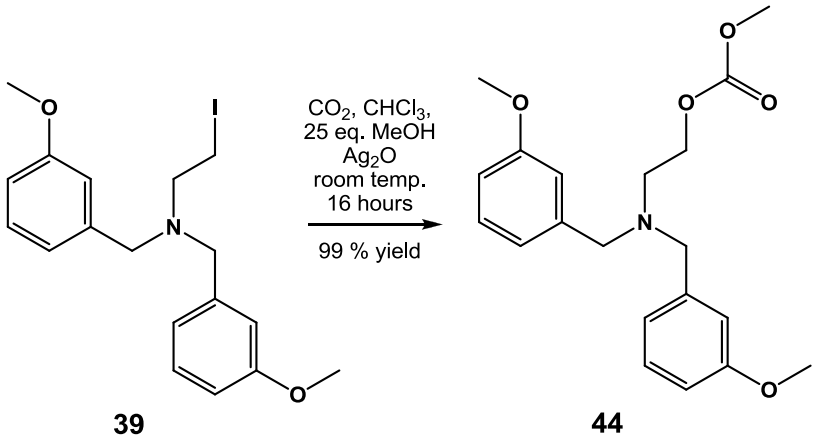


Scheme 23: Reaction under CO_2 to afford symmetrical carbonate **43**

2.1.3. Formation of Carbonates from Carbon Dioxide

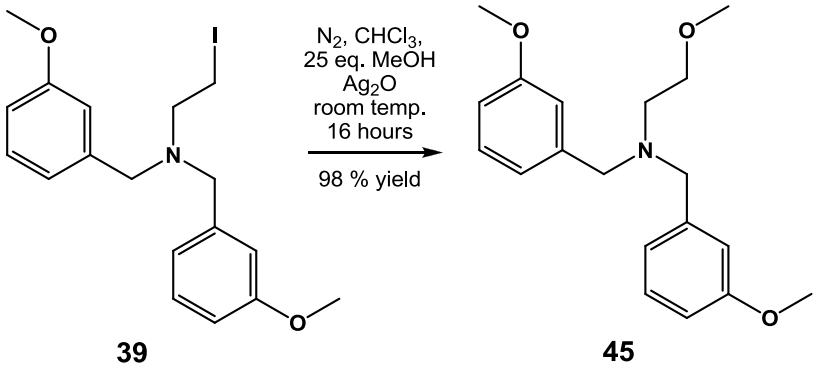
The reaction mechanism is difficult to understand due to the symmetry of the product **43** obtained therefore two reactions were attempted using a similar alkyl iodide but with the addition of methanol as an external nucleophile. One reaction was performed under a N_2 atmosphere and the other under a CO_2 atmosphere.

The reaction in the presence of CO_2 for 16 hours yielded carbonate **44** in 99 % yield (Scheme 24).



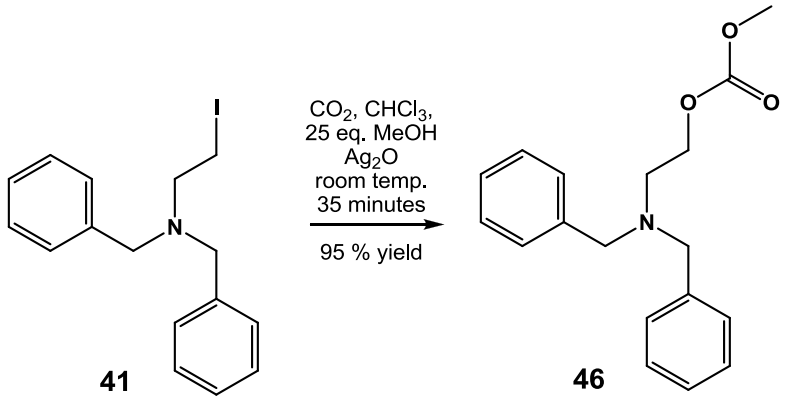
Scheme 24: Synthesis of carbonate **44** under a CO_2 atmosphere

The same reaction under a N_2 atmosphere yielded ether **45** in 98 % yield, which is simply the product from an S_N2 reaction (Scheme 25). These reactions demonstrate that atmospheric CO_2 is the likely source of the carbonyl group present in the product.

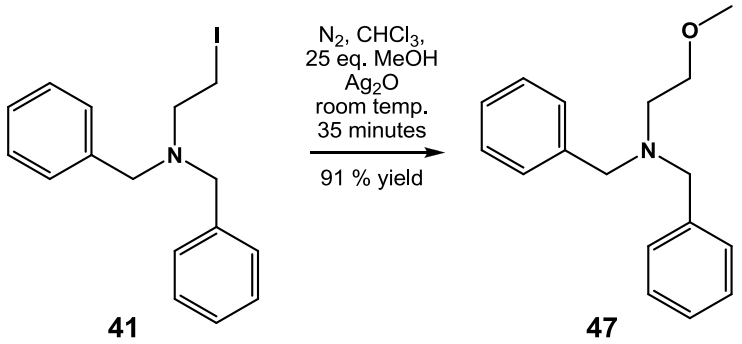


Scheme 25: Synthesis of ether **45** under a N_2 atmosphere

These same reaction conditions were attempted on unsubstituted iodide intermediate **41** and the corresponding carbonate **46** (Scheme 26) and ether **47** (Scheme 27) were also isolated in high yields after only 35 minutes.

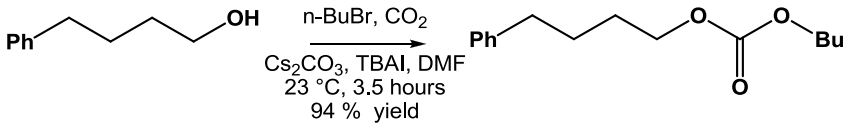


Scheme 26: Synthesis of carbonate **46** under a CO_2 atmosphere



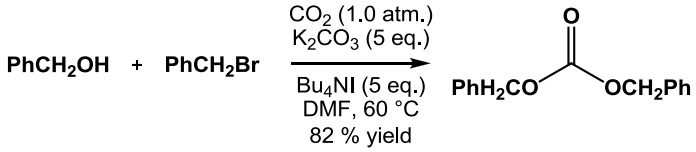
Scheme 27: Synthesis of ether **47** under N_2 atmosphere

This three-component coupling reaction occurs successfully combining a solid, liquid and a gas. Kim *et al.* previously reported a similar three-component coupling reaction in the presence of Cs_2CO_3 and CO_2 (Scheme 28).[66]



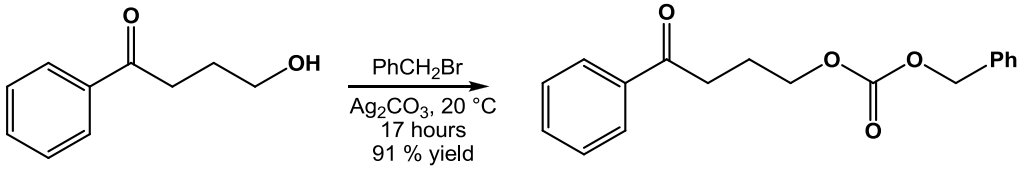
Scheme 28: Synthesis of carbonate using Cs_2CO_3[66]

In 2002, Shi and Shen reported a three-component coupling reaction of alcohols, CO_2 and alkyl halides in the presence of K_2CO_3 and nBu$_4$NI (Scheme 29).[67]



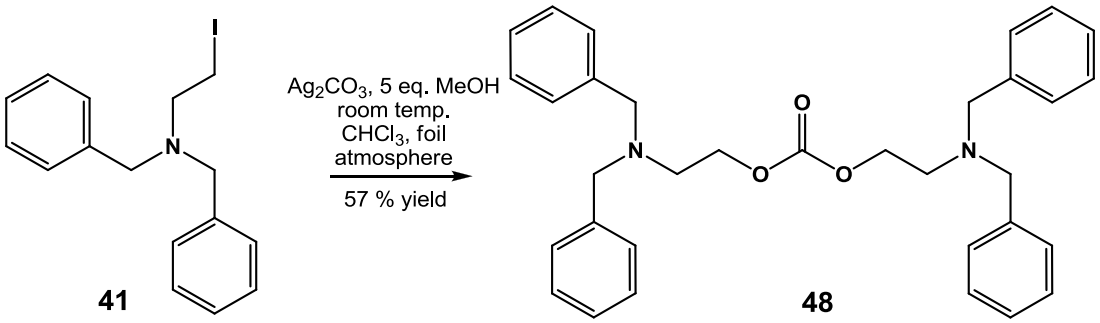
Scheme 29: Three-component coupling reaction to synthesise carbonate[67]

In 1994, Teranishi reported the preparation of carbonates using Ag_2CO_3 (Scheme 30).[68]



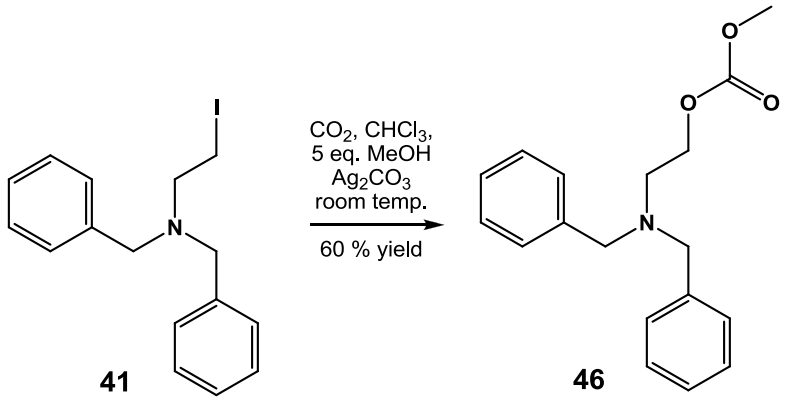
Scheme 30: Successful preparation for carbonates using silver carbonate[68]

Since the formation of Ag_2CO_3 is possible *in situ* (Ag_2O *(s)* + CO_2 *(g)* → Ag_2CO_3 *(s)*), we examined the effects of using pre-formed Ag_2CO_3 in the presence and absence of CO_2 to help further understand the mechanism of this reaction. Following conditions reported by Teranishi, Ag_2CO_3 and 5 equivalents of MeOH were added to a stirred solution of iodide intermediate **41** in $CHCl_3$ and the reaction was stirred at room temperature open to the atmosphere for 2.5 hours.[68] The unsymmetrical methyl carbonate **46** was not observed; instead the symmetrical carbonate **48** was obtained in 57 % yield (Scheme 31). This product is presumably formed by the displacement of the iodide twice and the carbonyl is incorporated from the silver carbonate.



Scheme 31: Synthesis of symmetrical carbonate **48** from Ag_2CO_3[68]

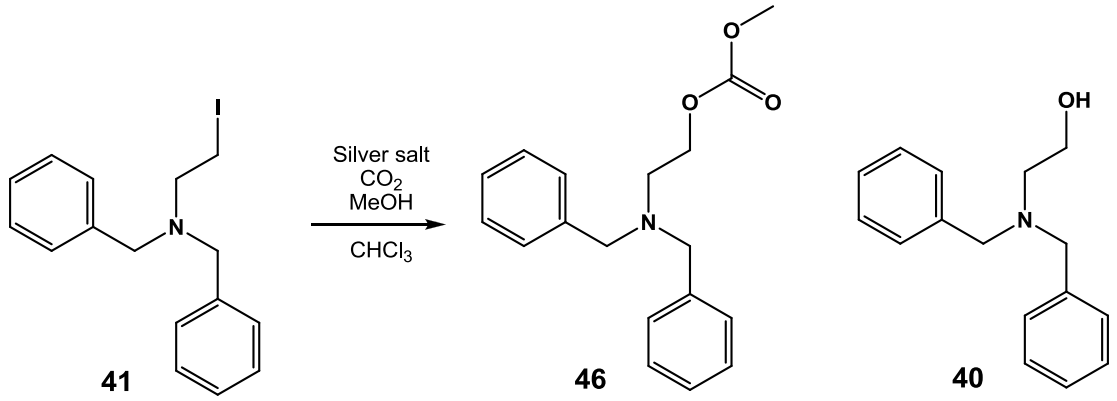
The same reaction conditions were performed under a CO_2 atmosphere to afford the unsymmetrical carbonate **46** in 60 % yield, which suggests the carbonyl from this reaction is incorporated from the gaseous CO_2 present in the reaction flask (Scheme 32)



Scheme 32: Synthesis of unsymmetrical carbonate **46** in the presence of Ag_2CO_3

The amount of MeOH and Ag_2O was investigated in the reaction, in addition to other silver salts (Scheme 33). These results are shown in (Scheme 34, Table 2 and Table 3).

A large excess of 25 equivalents of MeOH (1.00 % v/v) in the reaction gave 100 % conversion to the desired unsymmetrical carbonate **46** (Entry 3, Table 2). Lowering the equivalents of MeOH promoted the formation of both the symmetrical carbonate **48** and the alcohol **40** (Entries 1 and 2, Table 2). No alternative silver salts in this reaction gave any desired product (Scheme 34). 1.1 equivalents of Ag_2O gave the greatest yield of desired unsymmetrical carbonate **46** (Entry 1, Table 3).

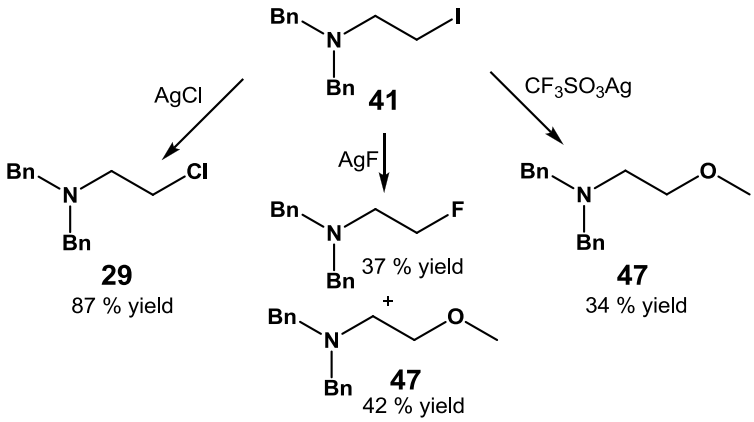


Scheme 33: Optimisation of reaction conditions to synthesise carbonate **46**

Entry	Eq. of MeOH	MeOH concentration (v/v %)	Ratio of unsymmetrical carbonate **46***	Ratio of alcohol **40***	Ratio of symmetrical carbonate **48***
1	1.1	0.04	0.65	0.13	0.22
2	5.0	0.20	0.69	0.22	0.09
3	25	1.00	1	0	0

*ratio obtained by ^{1}H NMR of crude reaction mixture

Table 2: Different equivalents of MeOH attempted in reaction conditions



Scheme 34: Attempted synthesis of carbonates using different silver salts with 25 eq. MeOH in $CHCl_3$ under an atmosphere of CO_2

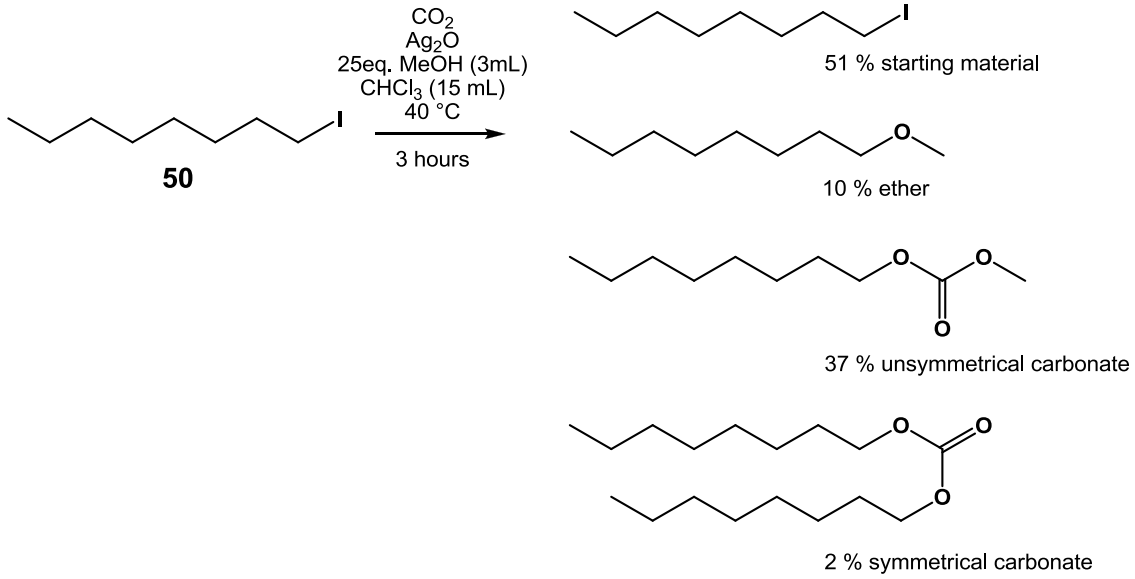
Entry	Eq. Ag_2O	% yield of **46**
1	1.1	95
2	0.7	80
3	0.5	67

Table 3: Different equivalents of Ag_2O to synthesise carbonate **46**

The optimised conditions to afford desired carbonate **46** are shown in Scheme 26, as any variations from these reaction conditions diminished the yields of the reaction.

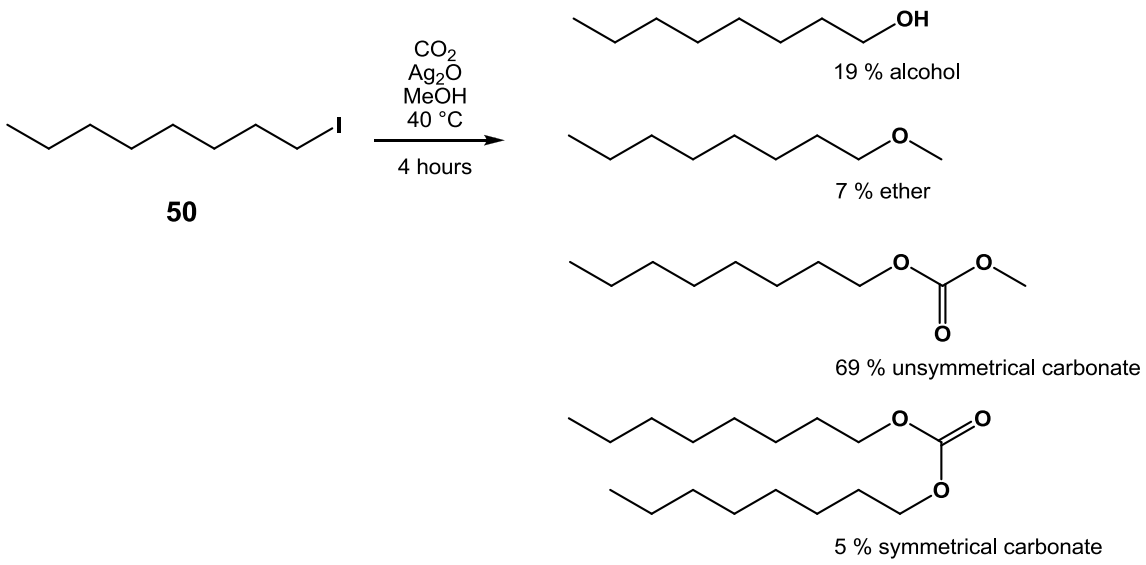
To date, reactions have been performed on functionalised iodides with possible neighbouring group participation from the nitrogen. To explore whether these optimised reaction conditions occur on unfunctionalised iodides, 1-iodooctane **50** was submitted to the optimised reaction conditions (Scheme 35).

The reaction occurred at a much slower rate and mixtures of compounds were observed by ^{1}H NMR of the crude reaction mixture. The major product in this reaction was the unsymmetrical carbonate in 37 %.



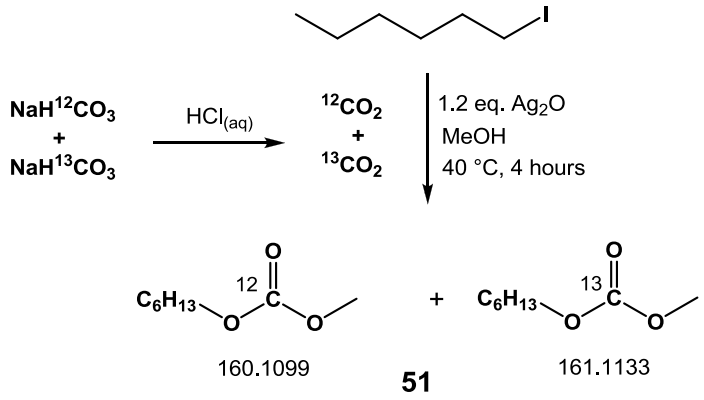
Scheme 35: Mixture of compounds observed by ^{1}H NMR of crude reaction mixture

The same reaction conditions were attempted in neat MeOH as the solvent, which improved the ratio of unsymmetrical carbonate observed by ^{1}H NMR in the crude reaction mixture to 69 % (Scheme 36).



Scheme 36: Mixture of compounds observed by ^{1}H NMR of crude reaction mixture (MeOH as solvent)

These results indicate that the carbonyl group originates from the gaseous CO_2 present in the reaction vessel. To demonstrate the ability of the Ag_2O-mediated reaction to capture gaseous CO_2 and transform it into functionalised carbonates, the reaction with ^{13}C-enriched CO_2 was performed (Scheme 37).



Scheme 37: ^{13}C-labelled CO_2 experiments to afford hexyl methyl carbonate **51**

Either 100 % $^{12}CO_2$ or 100 % $^{13}CO_2$ were generated by adding 3M $HCl_{(aq)}$ to either 5 equivalents of $NaH^{12}CO_3$ or $NaH^{13}CO_3$ and bubbling the CO_2 evolved directly through the reaction mixture and into an empty balloon. After 4 hours, the reaction mixture was filtered to remove the silver salts and evaporated under reduced pressure to afford the hexyl methyl carbonate **51** without the need for further purification.

Yield of 51 (%)	Measured ratio		Observed ratio ^{13}C NMR		Observed ratio HRMS	
	^{12}C	^{13}C	^{12}C	^{13}C	^{12}C	^{13}C
68	100	0	100	0	100	0
60	0	100	2.1	97.9	2.9	97.1

Table 4: Results of ^{13}C labelled CO_2 experiments

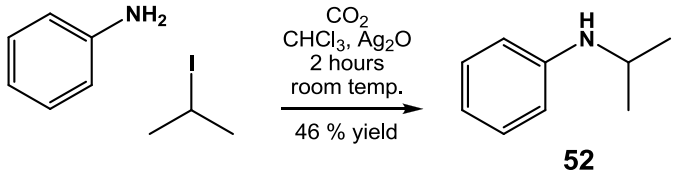
The yields of the methyl carbonate **51** vary between 60 % and 68 % which is a reflection of the heterogeneity of this reaction (Table 4). The reactants are in three different phases; gas (CO_2), liquid (solution of Hex-I in MeOH) and solid (insoluble Ag_2O).

The product was analysed by both ^{13}C NMR and High Resolution Mass Spectrometry. The integration of the carbonyl peak in the ^{13}C NMR spectrum provides information on the incorporation of ^{13}C using a simultaneous equation calculation (shown in appendices section **8.1.1.**). Due to different relaxation times, the integral of the carbonyl peak differs to the integrals of the remaining peaks in the NMR spectra. From the background ^{13}C NMR spectra, we can determine the difference in the integrals, which is incorporated in the simultaneous equation calculation as the "integration factor". Also, the carbonyl signal for the ^{13}C compound (**x**) will be 100 % abundant, so in order to make a direct comparison with the carbonyl signal for the ^{12}C compound (**y**), we must use a conversion factor which accounts for the natural abundance of ^{13}C (1.11 %). For all other C signals, **x** + **y** will simply equal the integral of that peak.

A ratio between the ^{12}C and ^{13}C products could also be determined from integration of the corresponding peaks obtained by High Resolution Mass Spectrometry (See calculations in appendices **8.1.1.**). The m/z of 100 % ^{12}C compound shows the MNa^+ ion at 183.1035 (39,090, 91.26 %) and 184.1067 (3,746, 8.74 %), due to the natural abundance of ^{13}C present in carbonate **51**. Using this background reading, the ratios of the mixtures were calculated. The proportion of the peak at 184.1 due to the natural abundance of ^{13}C is (3 746/39 090) of the peak at 183.09.

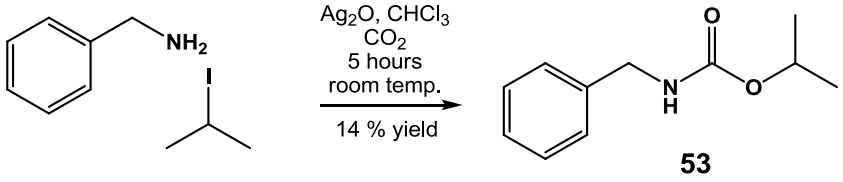
The results shown in Table 4 illustrate that the carbonyl is generated from the CO_2 in the reaction mixture and that it is possible to generate ^{13}C-labelled carbonates specifically labelled at the carbonyl group from $^{13}CO_2$ under mild reaction conditions.

To investigate this reaction further, amines were used as an alternative nucleophile. Both aniline and benzylamine were combined with 2-iodopropane to afford secondary amine **52** in 46 % yield, formed from a simple displacement reaction (Scheme 38). The desired carbamate was not obtained, possibly due to the lack of nucleophilicity of the amine.



Scheme 38: Formation of secondary amine **52**

Carbamate **53** was synthesised from benzylamine in a 14 % yield (Scheme 39). No other products were isolated; however, the low yields could possibly be caused by a competing displacement reaction.



Scheme 39: Synthesis of carbamate **53** under a CO_2 atmosphere

2.1.4. Applications

CO_2 absorption from the atmosphere could be a crucial factor in helping to reduce the effect of global warming. CO_2 is an attractive sustainable renewable carbon resource in organic synthesis as it is abundant and non toxic. The chemical fixation of CO_2 into organic molecules has generated a lot of interest over the past two decades due to its potential environmental benefits.[69]

Organic carbonates have been used for many years in a wide range of applications.[70] These include polymer science,[71] medical applications[72] and as protecting groups in synthetic chemistry. Carbonates are most commonly synthesised by the use of phosgene,[73] its

derivatives, or carbon monoxide.[74] Phosgene provides a simple high-yielding route towards the formation of carbonates; however, phosgene is a highly toxic reagent and therefore is not ideal. Dimethyl carbonate can be synthesised by carbonylation of methanol; however, this also involves the use of a toxic gas, carbon monoxide.[75] This therefore provides a demand for developing novel and safe procedures.

Many cancer treatments are not selective and target DNA in both healthy and cancerous cells, causing damage to proliferating healthy cells within the body. One strategy to provide an increase in the efficacy of these drugs is the development of prodrugs which are non-toxic.[76] Prodrugs are transformed into their corresponding biologically active compounds after administration, either by metabolism or chemical breakdown.

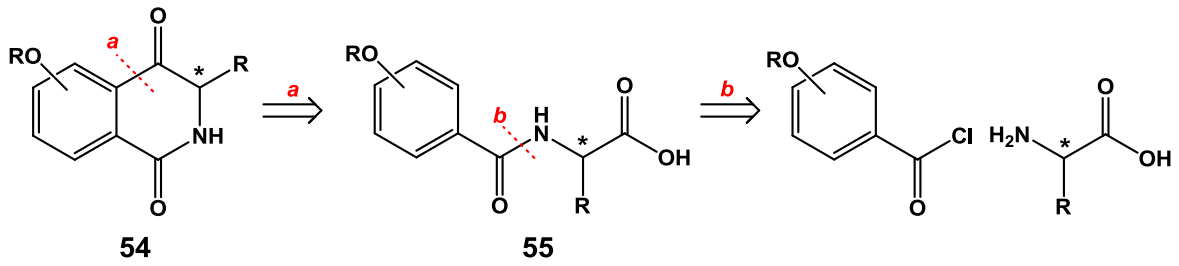
The three-component coupling reaction in the presence of Ag_2O to synthesise carbonates could potentially be applied to the synthesis of ^{13}C-labelled carbonate prodrugs. The use of $NaH^{13}CO_3$ would trap the labelled CO_2 in a known position and one of the most effective ways to follow drug metabolism is to use site-specific labelling.

The proposed synthetic route (disconnection shown in Scheme 7) to synthesise 1,2,3,4-tetrahydroisoquinolines *via* alkylation was unsuccessful. An alternative more favourable reaction which may access this framework could be an intramolecular Friedel-Crafts acylation reaction, which is now discussed. (see section **2.2**.).

2.2. Proposed Synthesis *via* Friedel-Crafts Acylation

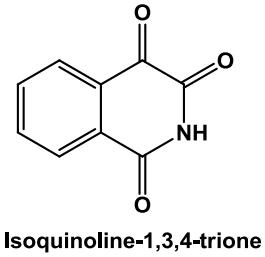
2.2.1. Proposed disconnection approach

Lactam **54** could be synthesised *via* a Friedel-Crafts acylation reaction following disconnection at position ***a*** to afford intermediate **55** (Scheme 40). Intermediate **55** can be accessed *via* a Schotten-Baumann reaction following disconnection at position ***b***, to furnish two readily accessible starting materials, an acid chloride and an amino acid.



Scheme 40: Disconnection approach to synthesise compound **54**

Chen *et al.* synthesised isoquinoline-1,3,4-trione which was found to inhibit caspase-3, a protein which plays a crucial role in cell apoptosis (Figure 14).[77]



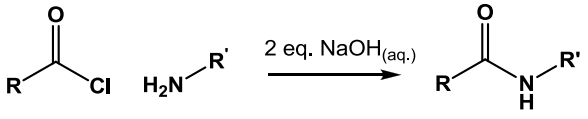
Isoquinoline-1,3,4-trione

Figure 14: Caspase-3 inhibitor isoquinoline-1,3,4-trione[77]

Lactam **54** could subsequently be reduced to potentially access a large range of analogues, using a variety of amino acid starting materials, which could possess biological activity.

2.2.2. Transformation

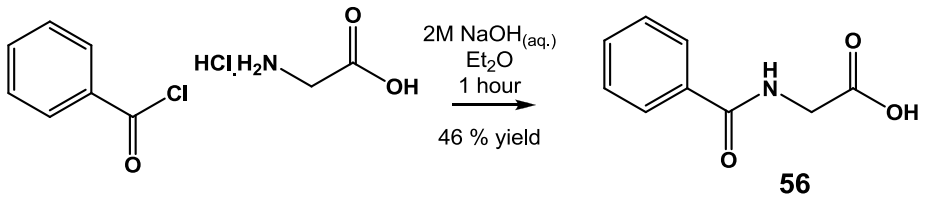
The Schotten-Baumann reaction is a biphasic reaction in which an amide is formed from an amine and acid chloride (Scheme 41). Two equivalents of base are required to prevent the acid produced in the reaction forming a salt with the remaining unreacted amine, resulting in a diminished yield. To optimise the reaction conditions, the second equivalent of aqueous base is added slowly during the reaction.



Scheme 41: Schotten-Baumann reaction

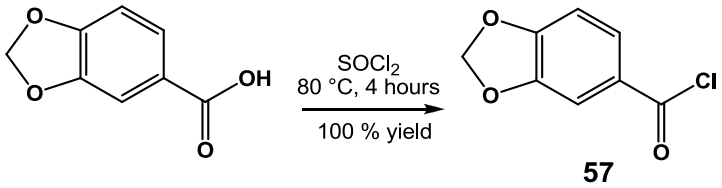
2.2.3. Synthesis of Intermediates

Following a procedure reported by Albrecht *et al.*,[78] benzoyl chloride and glycine gave desired intermediate **56** in a respectable 46 % yield (Scheme 42).



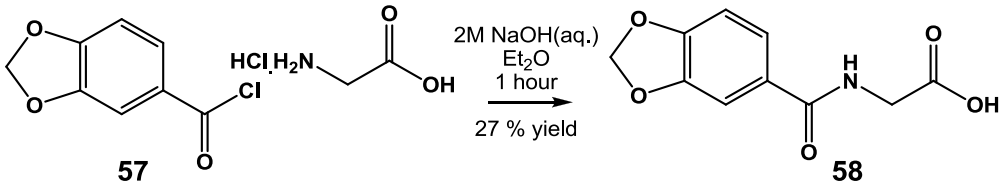
Scheme 42: Synthesis of intermediate **56**

To introduce functionality on the benzene ring, acid chloride **57** was synthesised using piperonylic acid and thionyl chloride at 80 ^{o}C for 4 hours (Scheme 43). Alternative reagents are oxalyl chloride and DMF in CH_2Cl_2, which also carried out this transformation. Thionyl chloride is much less easy to remove than oxalyl chloride, owing to its higher boiling point. However, it was the preferred method, as DMF was much more difficult to remove during work up.



Scheme 43: Synthesis of acid chloride **57**

Acid chloride **57** was then submitted to the same Schotten-Baumann conditions with glycine to afford intermediate **58** in a diminished 27 % yield (Scheme 44).



Scheme 44: Synthesis of intermediate **58**

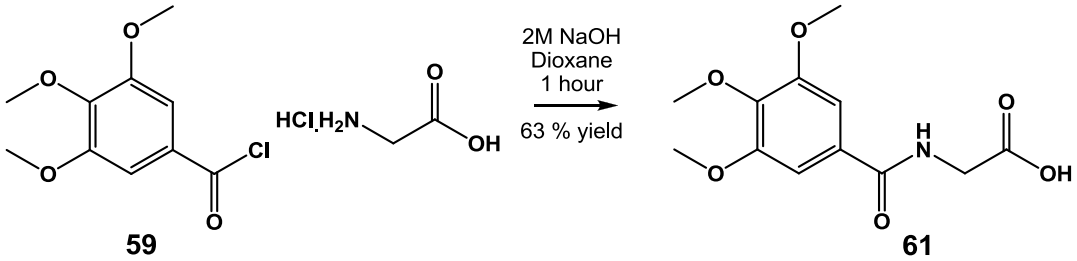
The reaction is performed in biphasic conditions, yet the acid chloride **57** still gives the desired products. By-products from this reaction could arise from hydrolysis of the acid choride which could be the reason for the discrepancy in yields of these two Schotten-Baumann reactions (Scheme 42 and Scheme 44).

Presumably increasing the rate of stirring could promote hydrolysis of the acid chloride which may be a reason for diminished yields. To explore this hypothesis, the synthesis of intermediate **58** was carried out but with alterations in the experimental procedure.

An initial reaction with extremely vigorous stirring was performed, followed by another more dilute reaction with additional Et_2O. An alternative solvent of CH_2Cl_2 was also investigated. All three gave the desired product **58** but in diminished yields. This suggests these reactions are extremely capricious due to the heterogeneity of the reaction mixture.

3,4,5-Trimethoxybenzoyl chloride **59** with glycine in Et_2O and aqueous NaOH at room temperature for 1 hour was unsuccessful. Only the starting material, 3,4,5-trimethoxy carboxylic acid **60**, was isolated from the reaction mixture, which is formed from hydrolysis of acid chloride **59**. The reason this reaction failed is due to the insolubility of 3,4,5-trimethoxybenzoyl chloride **59** in Et_2O. Identical reactions with either CH_2Cl_2 or acetone as the solvent, 3,4,5-trimethoxybenzoyl chloride **59** was again insoluble and only starting 3,4,5-trimethoxy carboxylic acid **60** was isolated from the reaction mixtures.

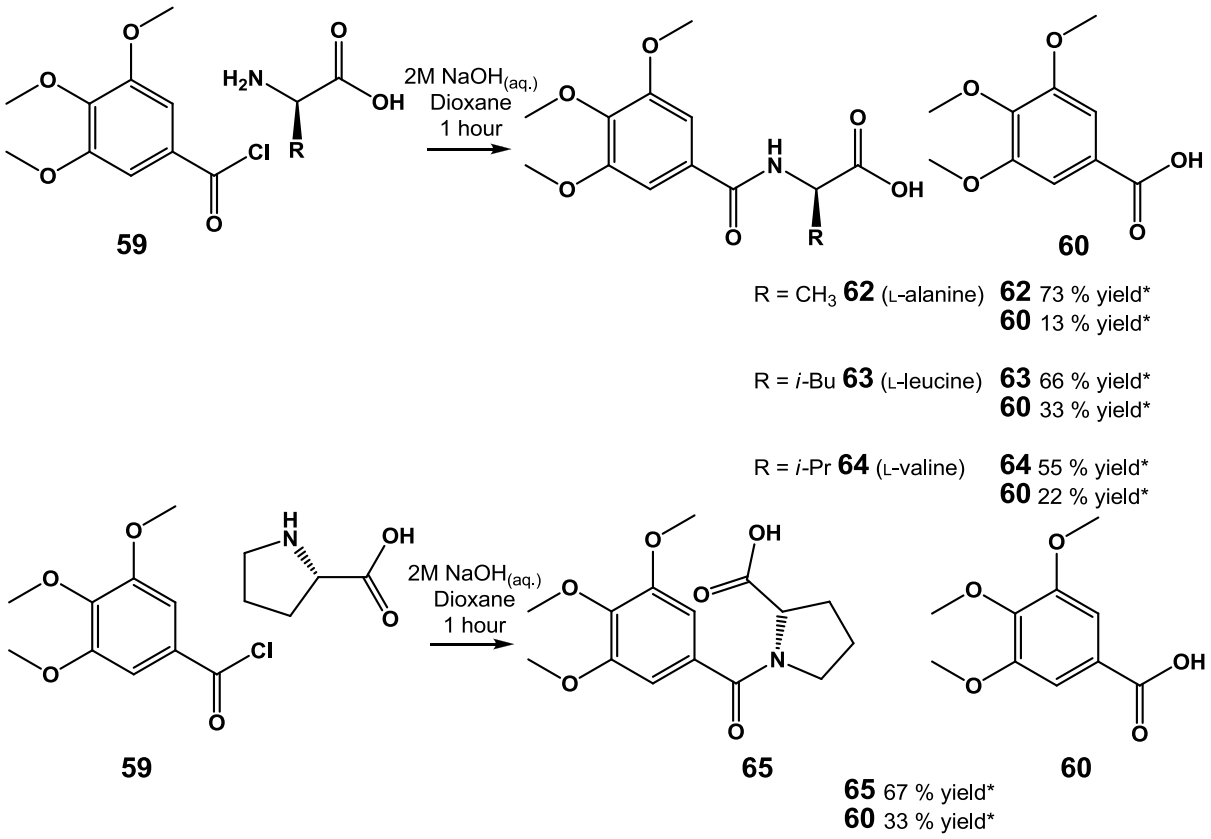
However, the use of 1,4-dioxane as a solvent, previously reported by Reeve and Pane, intermediate **61** was isolated in 63 % yield (Scheme 45).[79]



Scheme 45: Synthesis of intermediate **61** in 63 % yield[79]

Using 3,4,5-trimethoxybenzoyl chloride **59**, alternative amino acids were then investigated (Scheme 46). Previously the product precipitated upon the addition of $HCl_{(aq.)}$, however no precipitate was observed in these reactions and traditional extraction techniques were used. Different ratios of intermediates **62, 63, 64, 65** and also trimethoxybenzoic acid **60** were

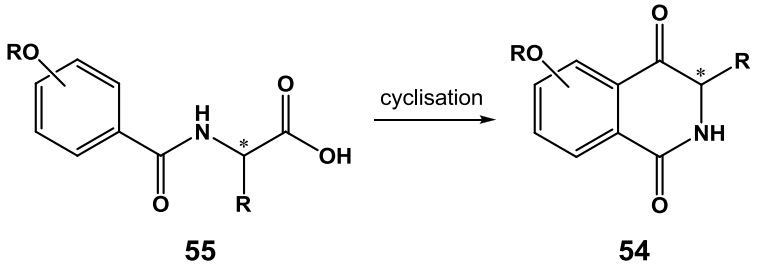
extracted from the reacton mixtures (Scheme 46). No modification in the equivalents of amino acid improved the yields or ratios of these reactions.



Scheme 46: Schotten-Baumann reactions - **yields calculated by ^{1}H NMR spectroscopy of crude reaction mixture*

2.2.4. Cyclisation of Intermediates

The Friedel-Crafts acylation reaction to cyclise the Schotten-Baumann intermediates will now be discussed (Scheme 47).



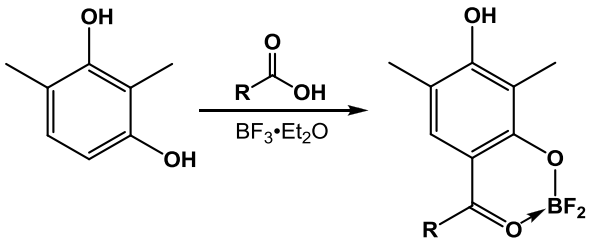
Scheme 47: Cyclisation of Schotten-Baumann intermediates

Intermediate **58** (Scheme 44) was subjected to thionyl chloride at room temperature to make the corresponding acid chloride, to investigate the Friedel-Crafts cyclisation. Unfortunately no desired cyclised product was seen by ^{1}H NMR spectroscopy and the reactants decomposed to give a mixture of unidentified compounds. The reaction was resubmitted to 50 ^{o}C heating in chlorobenzene for 16 hours to explore the possibility that the reaction was not yet complete. However, no identifiable products or cyclised material were present.

Cyclisation of intermediate **56** (Scheme 42) was attempted using a similar strategy to make the corresponding acid chloride, instead using oxalyl chloride and DMF in CH_2Cl_2 at room temperature for 1 hour. No desired cyclised product was observed and the starting material had decomposed.

The benzene ring with no substitution may not be nuceophilic enough to cyclise and therefore trimethoxy intermediate **61** (Scheme 45) was investigated. However, submitting **61** to oxalyl chloride and DMF in CH_2Cl_2 at room temperature for 1.5 hours gave a mixture of products and after column chromatography the desired cyclised product was not isolated.

Nicolaou *et al.* reported a Friedel-Crafts acylation through the activation of a carboxylic acid with neat $BF_3{\cdot}OEt_2$ (Scheme 48).[80]

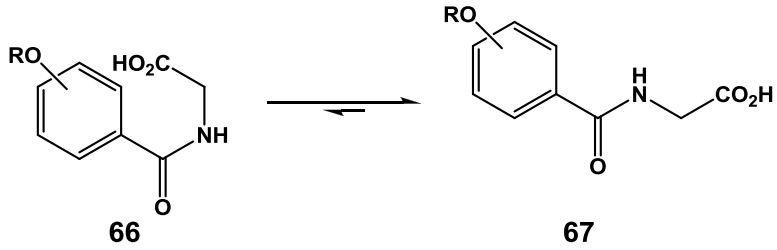


Scheme 48: $BF_3.Et_2O$ complex[80]

Following the procedure reported by Nicolaou *et al.*[80] the treatment of trimethoxy intermediate **61** with neat $BF_3{\cdot}Et_2O$ at 120 ^{o}C for 1 hour afforded no desired product. Friedel-Crafts cyclisation procedures have been reported by Ramana and Potnis[81] and Jia *et al.*[82] using H_2SO_4 at 90 ^{o}C and room temperature. Intermediate **61** was subjected to these reaction conditions but no desired product was observed.

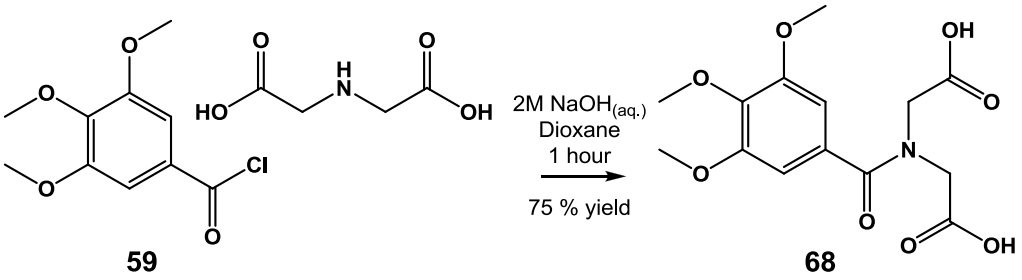
Activating the carboxylic acid intermediate **61** using TFAA and/or TFA was also explored; again no desired product was observed, although Barlow and Walsh reported a similar transformation in PPA with gentle heating over a water bath for 30 minutes.[83] Following this procedure on trimethoxy intermediate **61,** only starting material was also isolated.

An explanation for the overall unsuccessful Friedel-Crafts cyclisation of these acids could be due to the incorrect orientation. The amide prefers to sit in the *trans* conformer **67** rather than the *cis* conformer **66**. The *trans* conformer **67** forces the acid away from the nucleophilic aromatic ring, thus preventing the intramolecular cyclisation from occurring (Scheme 49).



Scheme 49: Amide conformations

An approach to overcome this is to synthesise di-acid intermediate **68**, which lacks this problem as the second carboxylic acid group is always available to react with the aromatic ring promoting the Friedel-Crafts acylation reaction (Scheme 50). Under the conditions reported by Reeve *et al.*[79] using 1,4-dioxane as solvent, di-acid **68** was synthesised in 75 % yield (Scheme 50).

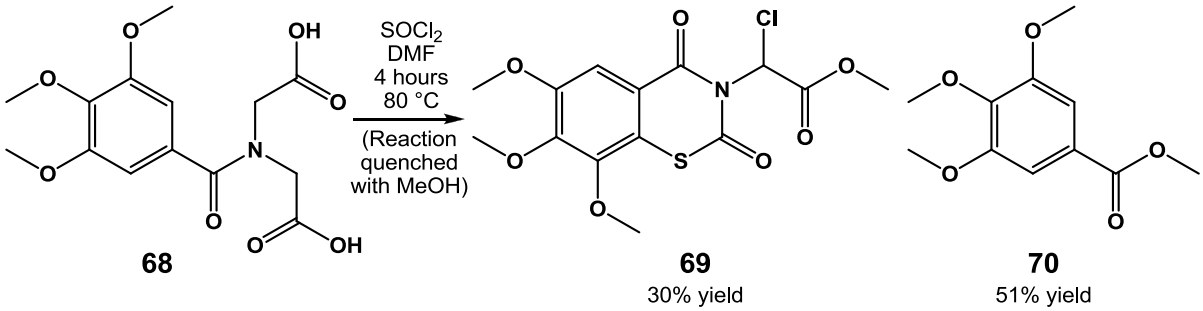


Scheme 50: Synthesis of intermediate **68** in 75 % yield

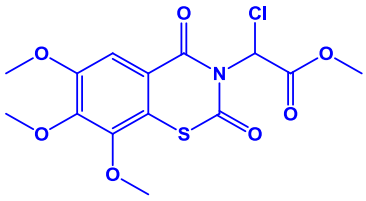
To explore the cyclisation of intermediate **68**, the di-acid was submitted to thionyl chloride with DMF at 80 ^{o}C for 4 hours and two products were isolated. After purification by column

chromatography, methyl 3,4,5-trimethoxybenzoate **70** was isolated in 51 % yield (Scheme 51).

Furthermore a second unknown compound was isolated and after thorough examination of the analytical data, the structure was still unclear. To clarify the structure of this mysterious product, a crystal structure was collected (Figure 15, See appendices **8.1.2.**), which successfully resolved the structure and matched the analytical data when assigned to compound **69** (Figure 15 and Scheme 51).



Scheme 51: Unexpected compounds **69** and **70** from $SOCl_2$ cyclisation reaction



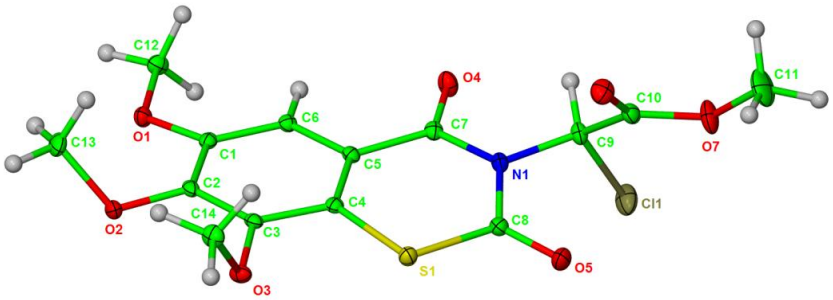
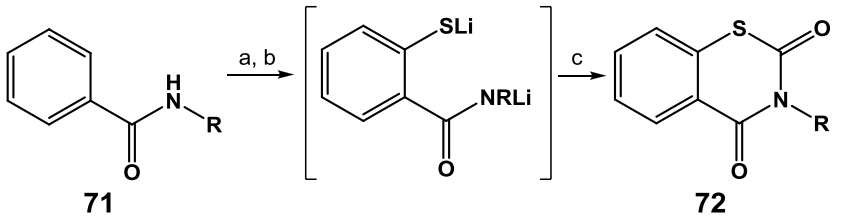


Figure 15: Crystal structure of compound **69**

The formation of cyclic product **69** was unexpected. The solid state structure of compound **69** shows the incorporation of a sulfur and chlorine atom from the thionyl chloride as well as the presence of a methyl ester. The methyl esters present in products **69** and **70** are formed by the addition of MeOH used to quench the reaction mixture. This reaction in thionyl chloride to synthesise compound **69** was, unfortunately, not reproducible and, when these reaction conditions were repeated, only higher yields of the methyl ester **70** were isolated in all cases.

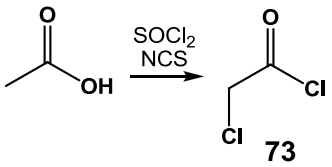
Wright previously synthesised a similar molecular scaffold in a one-pot procedure through the use of ortho lithiation (Scheme 52).[84] Benzamide **71** was treated with *n*-BuLi in THF at 0 ^{o}C for 1 hour, followed by the addition of sulfur powder. The reaction was heated at 50 ^{o}C until the starting material was consumed and the reaction was quenched into dilute acetic acid. The mixture was cooled thoroughly in ice and phosgene dissolved in toluene was added. After 24 hours products **72** were isolated in good yields (56 - 74 %).



a) n-BuLi, THF - 78 °C to 0 °C, b) sulfur powder, 50 °C, c) Cl_2C=O, 0 °C

Scheme 52: Compound **72** was previously synthesised using a one pot procedure[84]

The mechanism of this unexpected transformation (Scheme 51) was then explored. O'Hagen *et al.* reported the transformation of acetic acid to chloroacetyl chloride **73** in the presence of thionyl chloride and *N*-chlorosuccinimide (Scheme 53).[85]

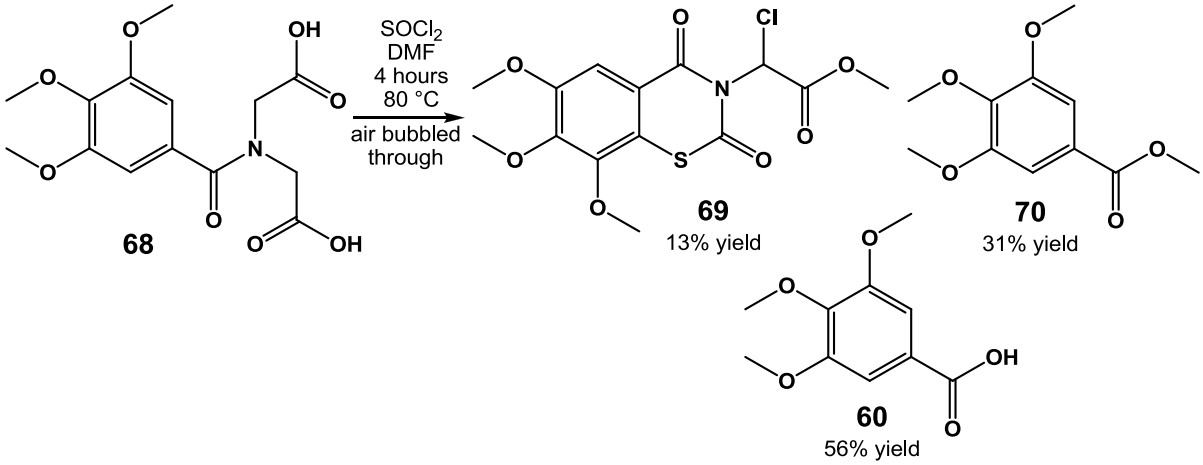


Scheme 53: Synthesis of chloroacetyl chloride **73**[85]

Due to the irreproducibility of this reaction, the procedure reported by O'Hagan *et al.* was attempted with acid **68**.[85] Acid **68** in thionyl chloride and NCS at 85 ^{o}C for 90 minutes gave no desired product **69** by crude ^{1}H NMR, only a mixture of unidentifiable compounds. The

mechanism also must consider the sulfur from thionyl chloride which has been incorporated into the structure. The mechanism of this reaction could possibly incorporate a Pummerer-type rearrangement.

Due to the irreproducible nature of these reactions the role of other factors such as the presence of air were investigated. The previous reaction conditions were repeated with air bubbled through the mixture at the start of the reaction. The reaction mixture was then heated at 80 ^{o}C for 4 hours and the desired product **69** was isolated in a diminished 13 % yield (Scheme 54), the major product of the reaction was trimethoxybenzoic acid **60,** which was isolated in 56 % yield.

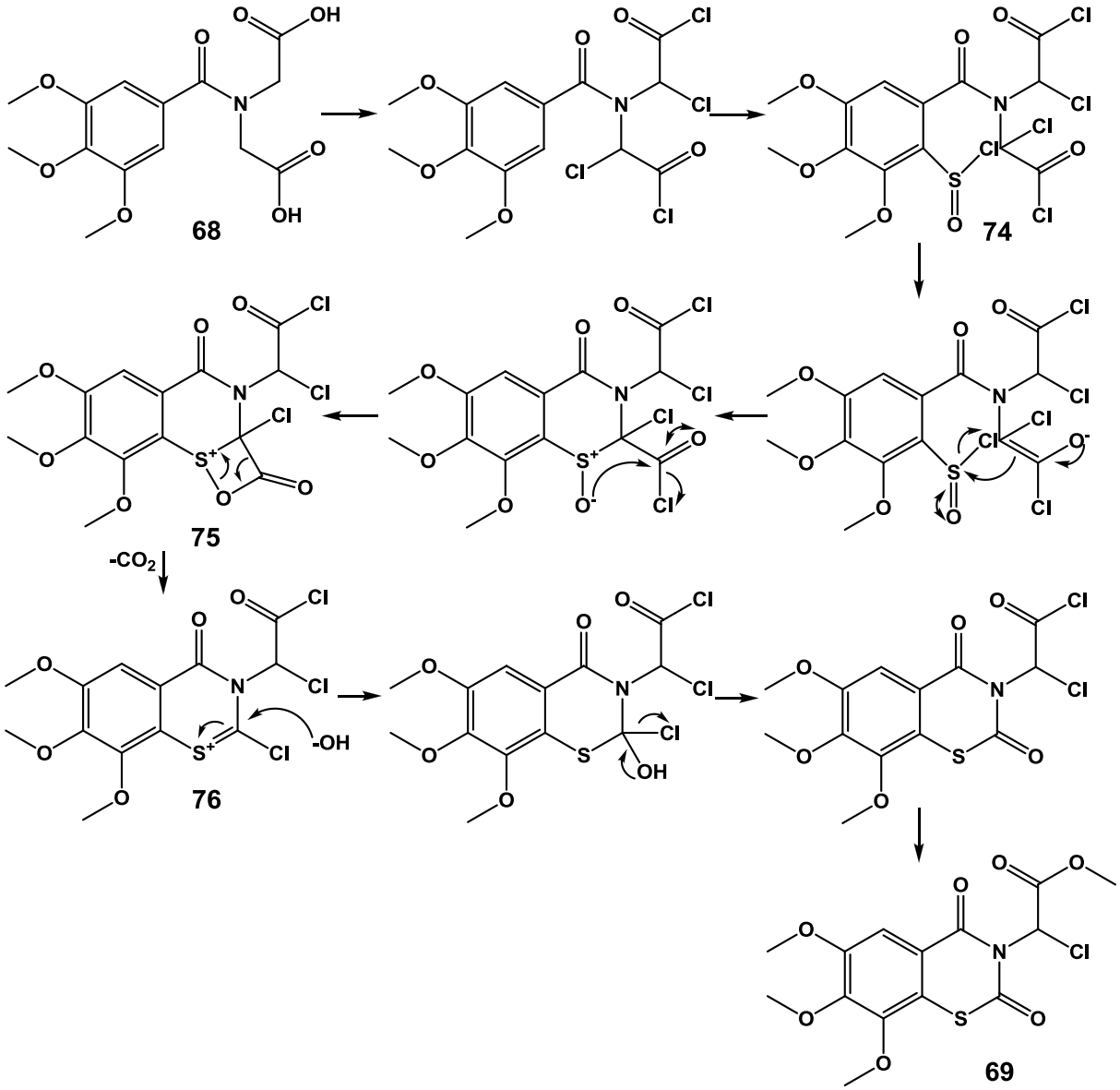


Scheme 54: Synthesis of compound **69** from acid **68** in thionyl chloride and DMF at 80 ^{o}C for 4 hours, open to the atmosphere

Combining the possibility of chlorinating at the α position (Scheme 53), the improvement in yield upon the addition of air (Scheme 54) and a Pummerer-type rearrangement, the proposed mechanism is shown (Scheme 55).

The proposed mechanism incorporates the double chlorination on both carboxylic acid groups, followed by nucleophilic attack of the electron-rich aromatic ring on $SOCl_2$ to afford intermediate **74**. Cyclisation followed by intramolecular attack of the sulfoxide on the acid chloride forms the cyclic intermediate **75**, followed by the loss of CO_2. Structure **75** does seem unlikely, although the same effect could also be achieved if this step occured *via* intermolecular attack from the sulfoxide of another molecule, again followed by the loss of

CO_2 to result in the same compound **76**. Attack of -OH and loss of the chloride affords 1,3-thiazinanedione intermediate which is quenched with MeOH to form the observed product **69**. Further investigation is still required to determine the precise mechanism of this transformation and to subsequently optimise the reaction conditions.



Scheme 55: Proposed mechanism for the formation of compound **69**

2.3. Conclusions

The aim of the work described within this chapter was to synthesise the tetrahydroisoquinoline framework present in a wide range of natural products. The synthesis of this framework was initially attempted using a Friedel-Crafts alkylation reaction. Although the appropriate precursors were synthesised in good yields the Friedel-Crafts alkylation approach proved unsuccessful.

An unexpected but interesting carbonate side product was observed during the attempted Friedel-Crafts cyclisation reactions. This serendipitous result was due to the incorporation of CO_2 from the atmosphere in the presence of Ag_2O. A side project was pursued to explore this further as a potential synthesis for carbonate compounds. Mechanistic studies of the carbonate synthesis were conducted using ^{13}C-labelled CO_2. These studies proved that CO_2 present in the reaction vessel was indeed the source of the carbonyl moiety. A range of carbonates were subsequently synthesised using this novel approach in high yields.

Alternative, more favourable, Friedel-Crafts acylation reaction conditions were then explored to access the tetrahydroisoquinoline framework. This approach was also unsuccessful however, the use of $SOCl_2$ led to an interesting side product. Both a sulfur and chlorine atom, from the $SOCl_2$, were incorporated into the precursor to form a novel cyclic product, which was crystographically characterised. Preliminary mechanistic studies were performed and a mechanism was proposed.

3. Chapter Three - A/B Analogues of Dihydroisoquinolinones

Dihydroisoquinolinones were subsequently investigated as these analogues are also found in many naturally occuring compounds (Figure 16).

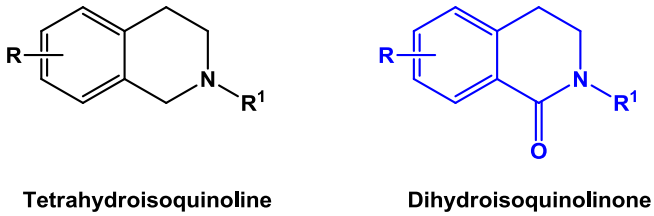


Figure 16: Structure of tetrahydroisoquinolines and dihydroisoquinolinones

3.1. Proposed Synthetic Route

The initial focus was to synthesise the A/B lactam core to make the simplified dihydroisoquinolinone framework of pancratistatin **16** and narciclasine **17** (Figure 17)

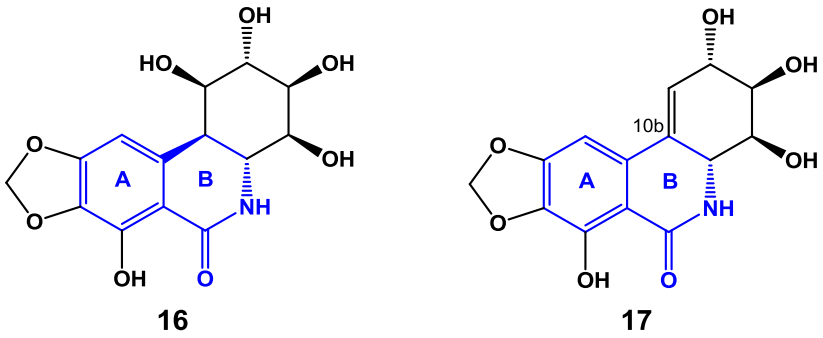
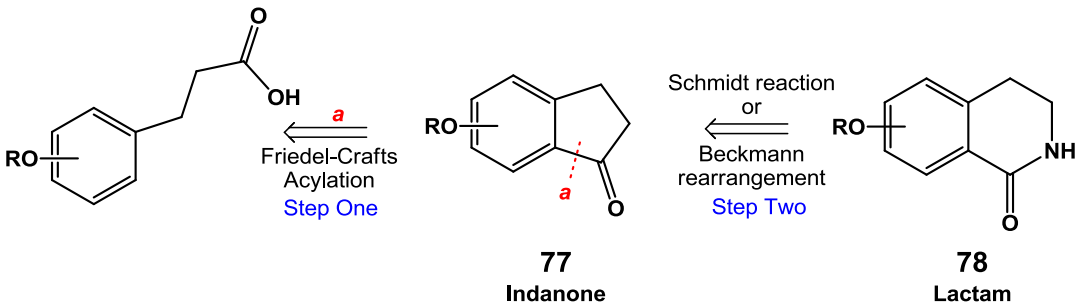


Figure 17: Pancratistatin **16** and narciclasine **17**

The proposed synthetic route to access the simplified A/B lactam core of pancratistatin **16** and narciclasine **17** involves two main transformations (Scheme 56). The lactam framework **78** will be accessed *via* an indanone intermediate **77** using a Schmidt reaction or Beckmann rearrangement (Step Two). The indanone intermediate **77** will be synthesised following disconnection at position ***a*** *via* a Friedel-Crafts acylation reaction on a substituted propanoic acid (Step One).



Scheme 56: Proposed disconnection approach to access the simplified A/B lactam core of pancratistatin **16** and narciclasine **17**

3.1.1. Step One - Indanones

The indanone is not only an important intermediate to gain access to the lactam core; it may also be of great interest itself as indanones have been shown to possess potential biological activity. Natural products containing the indanone framework have previously been isolated from the bark of *Taiwania cryptomerioides* and other related plants (Figure 18).[5] Both natural products Taiwaniaquinol B and D have been tested for their cytotoxicity against the KB epidermoid carcinoma cancer cell line.[86] Taiwaniaquinol B gave an IC_{50} value of >10 μM and Taiwaniaquinol D gave an improved IC_{50} value of 3.5 μM.

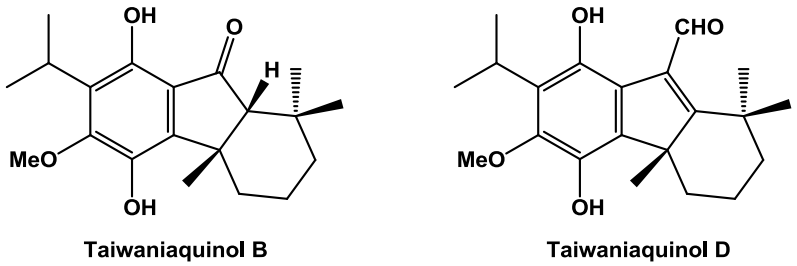


Figure 18: Taiwaniaquinol B and Taiwaniaquinol D[5]

Another compound which contains the indanone moiety and which has been shown to possess biological activity is indanocine **79** (Figure 19).[87] Indanocine has an IC_{50} value of 0.001 μM against the Jurkat cell line[87] and more recently has been shown to bind to microtubules selectively, inducing apoptosis in multi-drug resistant cancer cells.[88] Indanones are very attractive structures in their own right and the intermediates synthesised in this route may be of interest to pursue further.

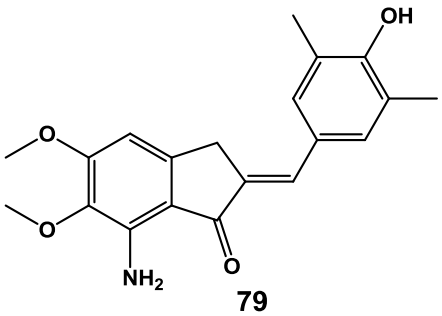
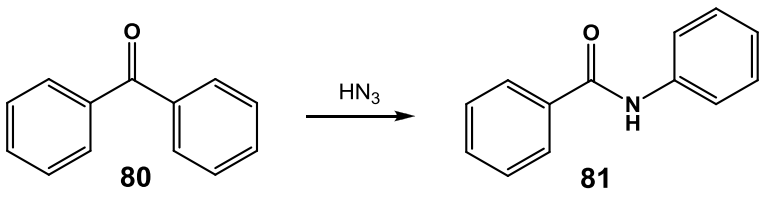


Figure 19: Indanocine **79**[87]

3.1.2. Step Two - Schmidt Reaction

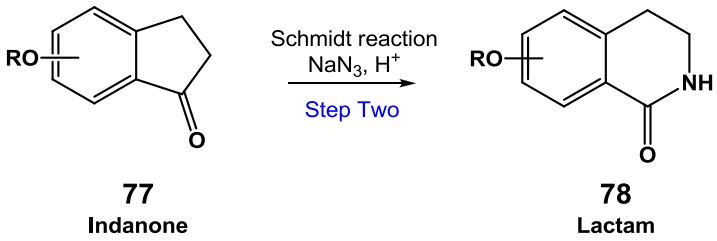
The second transformation in the proposed synthetic route is the formation of the lactam core from is corresponding indanone which could be achieved by a Schmidt reaction or Beckmann rearrangement.

The Schmidt reaction was first discovered in 1924 by Karl Friedrich Schmidt who reported the transformation of benzophenone **80** and hydrazoic acid to benzanilide **81** (Scheme 57).[89]



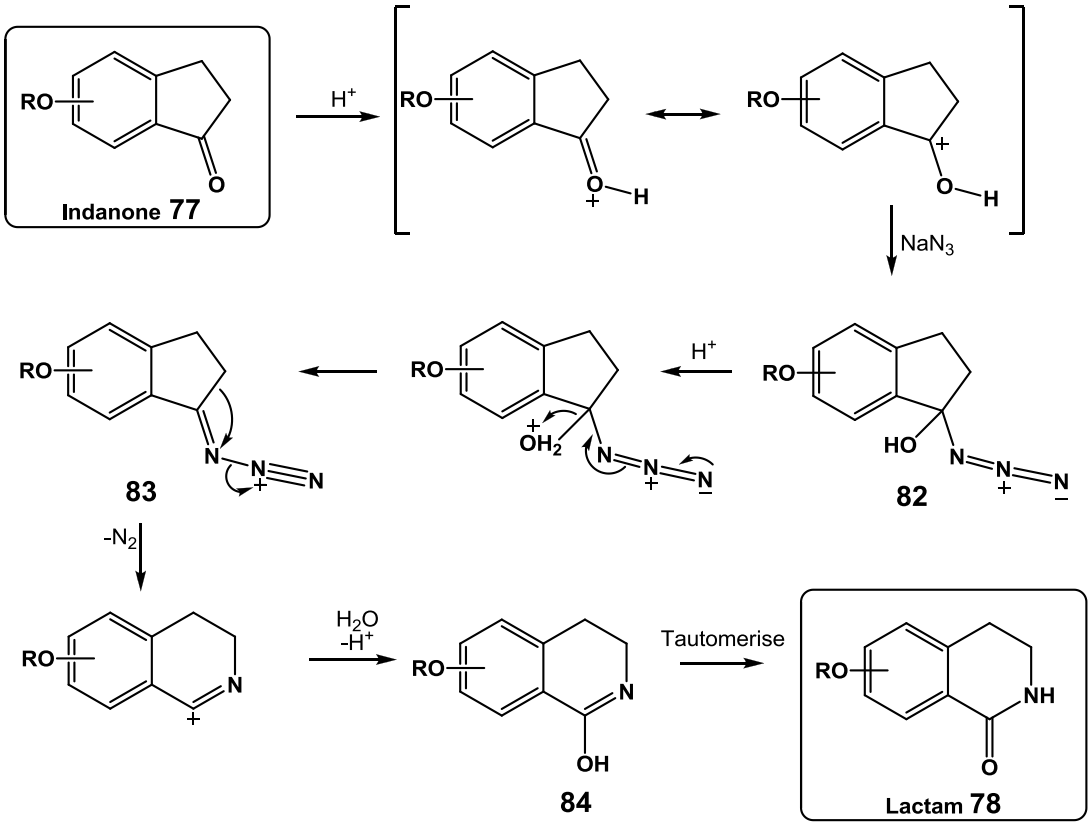
Scheme 57: The first reported Schmidt reaction on benzophenone **80** to afford benzanilide **81**[89]

When the indanone intermediate **77** is treated with sodium azide under acidic conditions, it should undergo a Schmidt reaction to yield the desired lactam **78** (Scheme 58).



Scheme 58: Schmidt reaction to yield desired lactam **78** from indanone **77**

The mechanism for the Schmidt reaction on a ketone (indanone **77**) starts with the activation of the carbonyl by protonation, followed by nucleophilic addition by the azide to form intermediate **82** (Scheme 59). Intermediate **82** then loses water in an elimination reaction to form imine **83**, which undergoes alkyl migration driven by the loss of nitrogen. The subsequent attack of water and loss of a proton affords **84**, which is the tautomer of the final amide (lactam **78**).



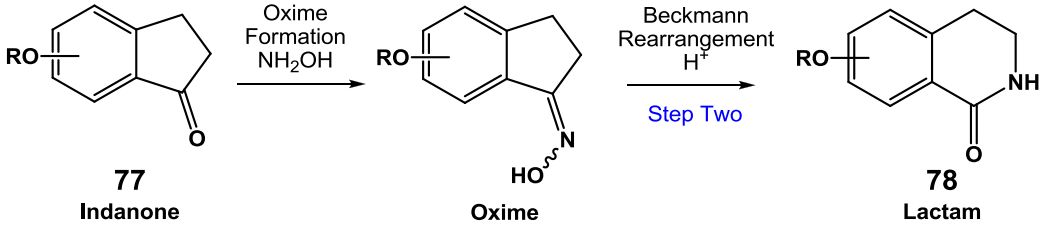
Scheme 59: Schmidt reaction mechanism

Imine **83** could also undergo aryl migration to afford the undesired lactam regioisomer **86**. Although the cation intermediate formed on elimination of the nitrogen *via* alkyl migration is more stabilised by the benzylic position, as shown in Scheme 61.

3.1.3. Step Two - Beckmann Rearrangement

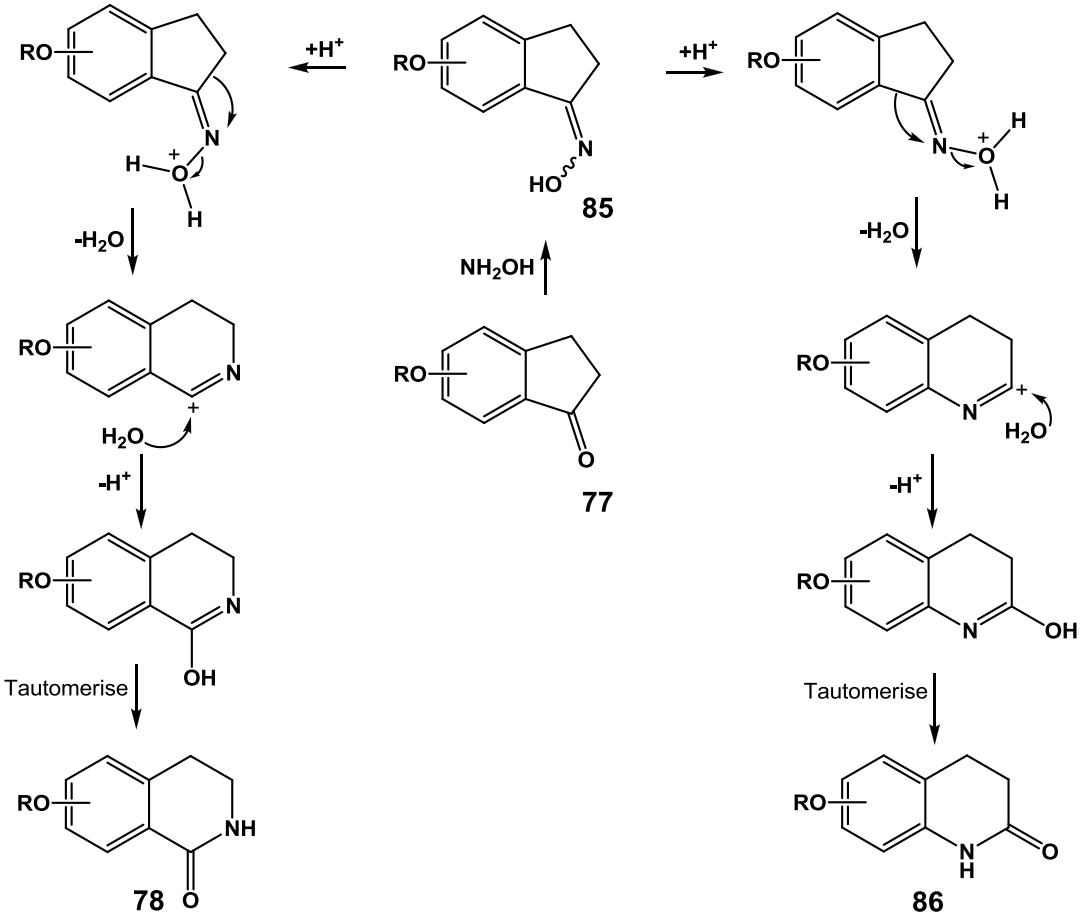
The same transformation could also be carried out using a Beckmann rearrangement on an oxime intermediate under acidic conditions (Scheme 60). The use of the Beckmann

rearrangement allows an alternative method to access the desired lactam framework **78** and is worth investigating.



Scheme 60: Beckmann rearrangement

The mechanism of this reaction is outlined in Scheme 61. Oxime **85** is initially formed, which under acidic conditions becomes protonated and migration of the group *trans* to the OH in the oxime occurs.



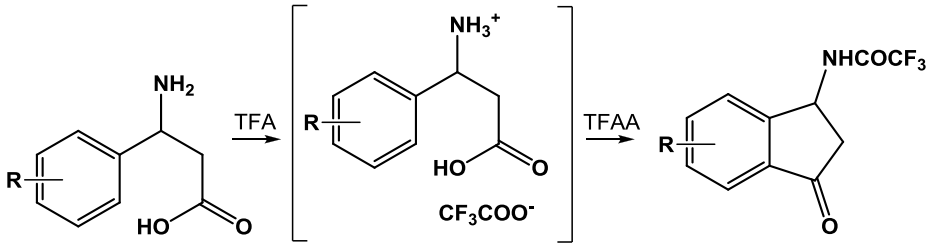
Scheme 61: Beckmann rearrangement mechanism

3.2. Results and Discussion

3.2.1. Step One - Intramolecular Indanone Synthesis

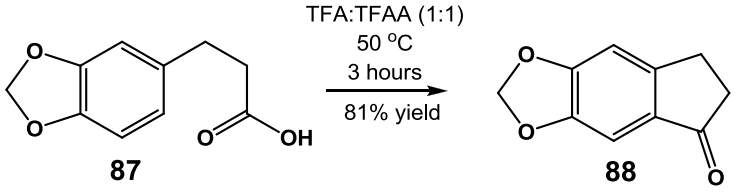
A/B analogues were initially used as a simplified framework and intramolecular indanone syntheses were attempted from substituted propanoic acids as starting materials.

Dallemagne *et al.* reported in 1991 a procedure for intramolecular cyclisation reactions, to generate indanones from their corresponding dihydrocinnamic acids, using trifluoroacetic acid (TFA) and trifluoroacetic anhydride (TFAA) (Scheme 62).[90]



Scheme 62: Intramolecular cyclisation using TFA/TFAA to afford indanones[90]

Following these reaction conditions, 3-(3,4-methylenedioxyphenyl)propanoic acid **87** was treated with TFA and TFAA at 50 ^{o}C for 3 hours. After aqueous work-up, the desired indanone **88** was isolated in 20 % yield. Without an aqueous work-up, column chromatography of the reaction mixture gave the desired indanone **88** in 81 % yield (Scheme 63). Unfortunately the results were not consistent so the reaction was explored further.

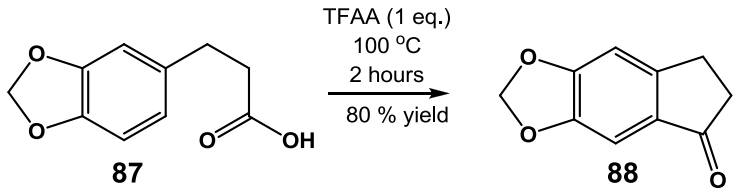


Scheme 63: Intramolecular A/B indanone synthesis of compound **88**

3-(3,4-Methylenedioxyphenyl)propanoic acid **87** was treated with TFA:TFAA (1:1) in a sealed pressure tube and heated to 100 ^{o}C for 1 hour. The isolation of indanone **88** gave a comparable 84 % yield, although it was difficult to isolate the products from the crude mixture by column chromatography due to by-products eluting closely.

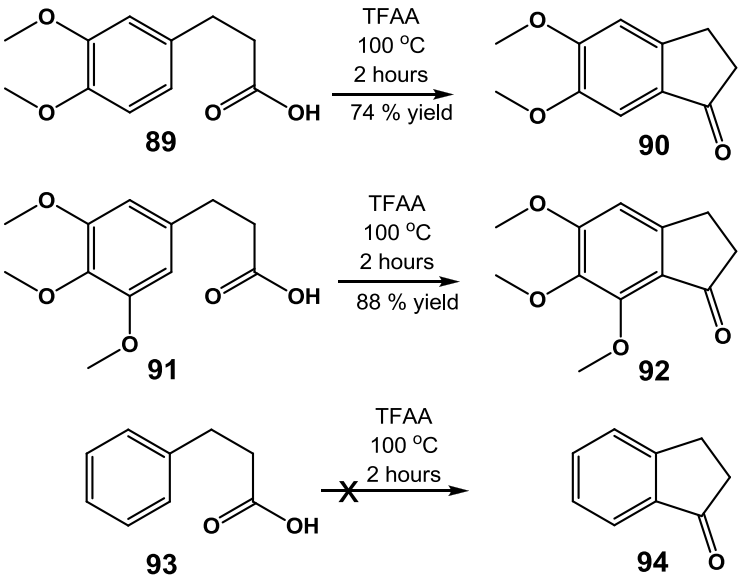
A similar transformation by Kolokythas *et al.* in 2006 was reported using TFA and TFAA in a (2:1) ratio.[91] This was attempted on acid **87** in TFA: TFAA (2:1) at 100 ^{o}C in a sealed pressure tube for 2 hours to yield 72 % of indanone **88**.

It was then investigated whether the addition of either TFAA or TFA alone would afford the indanone product. The reaction of acid **87** in 2 eq. of TFA at 100 ^{o}C for 2 hours gave only starting material. However, the reaction in 1 eq. of TFAA at 100 ^{o}C for 2 hours afforded 80 % yield of indanone **88** with the most effective purification (Scheme 64).



Scheme 64: Optimised conditions for intramolcular indanone synthesis

Other substituted 3-arylpropanoic acids were then subjected to these optimised conditions, as shown in Scheme 65.

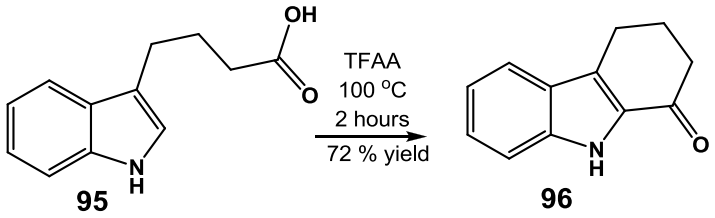


Scheme 65: Synthesis of intramolecular indanones **90** and **92**

3-(3,4-dimethoxyphenyl)propanoic acid **89** gave the corresponding indanone **90** in 74 % yield and 3-(3,4,5-trimethoxyphenyl)propanoic acid **91** afforded indanone **92** in 88 % yield.

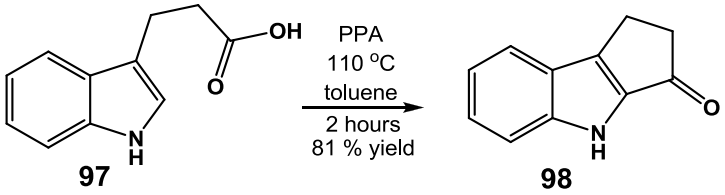
The reaction with hydrocinnamic acid **93** gave a complicated mixture of compounds and no desired product **94** was isolated, suggesting the aromatic ring carrying no activating methoxy groups may not be nucleophilic enough for this cyclisation to occur.

The scope of this reaction was explored further by subjecting 4-(indol-3-yl)butanoic acid **95** to the optimised reaction conditions, affording the desired cyclic ketone **96** in 72 % yield (Scheme 66). This transformation had previously been reported with PPA in toluene at 110 ^{o}C for 4 hours in 85 % yield.[92]



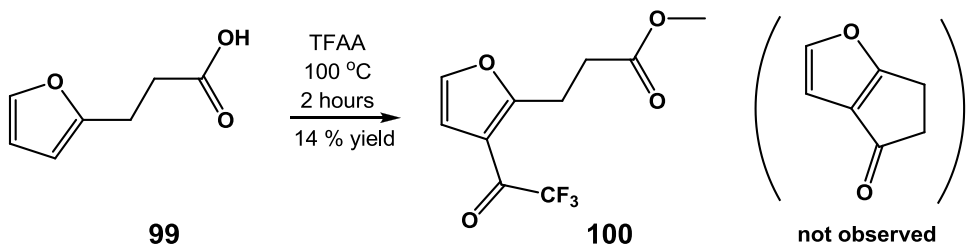
Scheme 66: Intramolecular Friedel-Crafts cyclisation reaction to synthesise cyclic ketone **96**

The reaction of TFAA with 4-(indol-3-yl)propanoic acid **97** proved unsuccessful, instead, a procedure reported by Maertens *et al.* in 2004 was used. Following this procedure, PPA was treated with 4-(indol-3-yl)propanoic acid **97** in toluene at 110 ^{o}C for 4 hours to yield the cyclic ketone **98** in 81 % yield (Scheme 67).[92]



Scheme 67: Intramolecular Friedel-Crafts cyclisation reaction to synthesise cyclic ketone **98**

3-(Furan-2-yl)propanoic acid **99** in TFAA at 100 ^{o}C in a pressure tube for 2 hours did not yield the desired corresponding cyclic ketone. Instead, the furanyl ring performed an intermolecular attack on TFAA to afford product **100** in 14 % yield and a mixture of other unidentified compounds (Scheme 68). The methyl ester was obtained possibly due to the presence of an anhydride intermediate in the reaction mixture, which upon treatment with MeOH to transfer the reaction mixture from the pressure tube afforded the methyl ester product **100**.



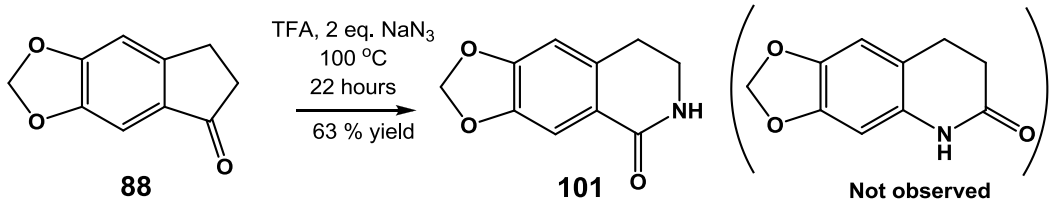
Scheme 68: The formation of side product **100** from 3-(furan-2-yl) propanoic acid

3.2.2. Step Two - Schmidt Reaction

The first transformation has been carried out under mild conditions and high yields to afford a range of A/B indanone derivatives. The Schmidt reaction or Beckmann rearrangement should now provide access to the A/B lactam core.

Schmidt reactions attempted on A/B indanone **88** using previously reported H_2SO_4 or PPA were unsuccessful. Since the indanone structures have previously been synthesised using TFA, the same conditions were attempted using TFA as the acid in the Schmidt reaction. When this reaction was performed, the desired lactam **101** was obtained in 32 % yield.

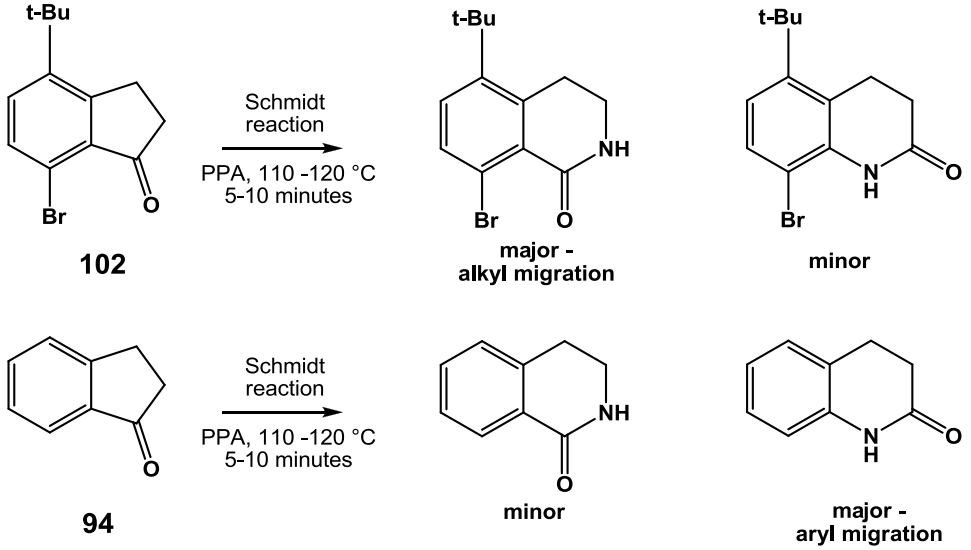
To optimise the conditions and improve this yield, the experiment was repeated with 2 eq. NaN_3 at 100 °C in TFA and left to stir for 22 hours in a sealed pressure tube, which gave lactam **101** in an improved 63 % yield (Scheme 69). This reaction yields N_2 gas as a side product and so safety measures were taken to prevent the danger of increasing pressure.



Scheme 69: Schmidt reaction on A/B indanone **88** to afford lactam **101** in 63 % yield

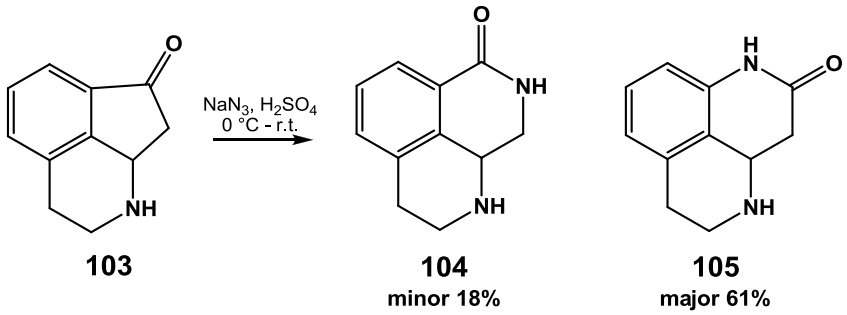
This same lactam **101** has previously been synthesised following a different route within the group. An identical analytical ^1H NMR spectrum confirmed that **101** is the observed regioisomer.[93]

In 1965, Lansbury and Mancuso reported that the Schmidt reaction of lactam **102** in PPA mainly gave the product of alkyl migration (Scheme 70).[94] However, using indanone **94** with no substitution, the major product was from aryl migration.



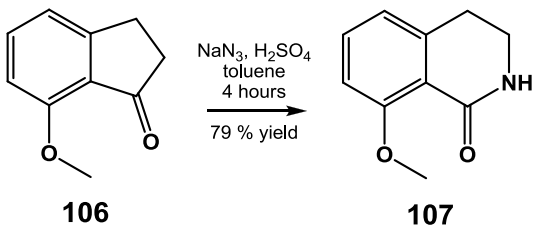
Scheme 70: Non-regiospecificity in the Schmidt reaction[94]

More recently, Torrisi *et al.* in 2010 reported a Schmidt reaction on indanone **103** and NaN_3 again in H_2SO_4 to afford also the product from aryl migration, lactam **105** in 61 % yield (Scheme 71).[95]



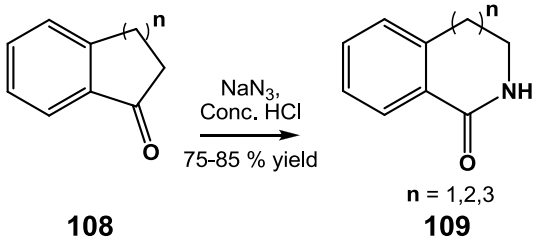
Scheme 71: Schmidt reaction on indanone **103** to afford lactams **104** and **105**[95]

In 2006, Jesudason *et al.* reported a Schmidt reaction on a similar A/B indanone **106** with a methoxy group *ortho* to the carbonyl group.[96] Using H_2SO_4, the major product was from migration of the alkyl group to afford the desired regioisomer, lactam **107** in 79 % yield (Scheme 72).



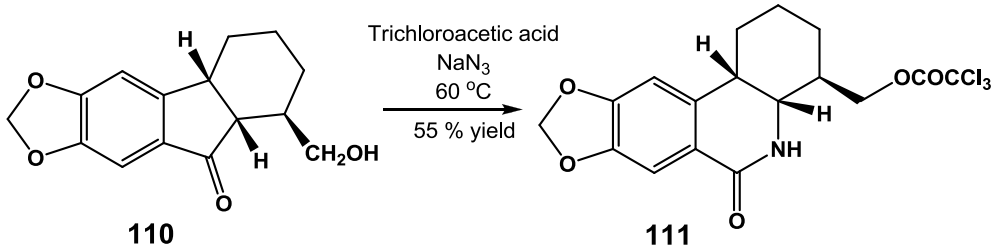
Scheme 72: Schmidt reaction on indanone **106** to synthesise lactam **107** in 79 % yield[96]

In 2009, Ortega *et al.* reported a Schmidt reaction on unsubstituted A/B indanones **108** (Scheme 73).[97] Schmidt reactions using PPA or H_2SO_4 have previously afforded lactams from aryl migration as the major product (Scheme 70 and 71). However, Ortega *et al.* found that, by changing the reaction medium to concentrated aqueous HCl, lactams from alkyl migration **109** are afforded as the major product in 75-85 % yields (Scheme 73).



Scheme 73: An example of a Schmidt reaction using conc. aq. HCl as the acid catalyst[97]

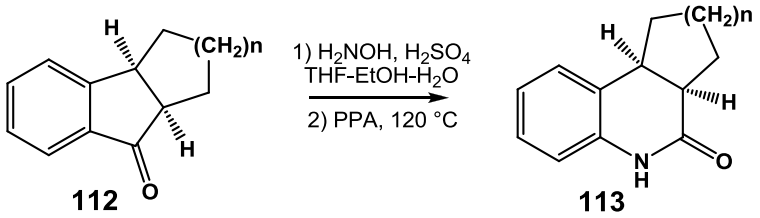
A previous report with a similar substrate containing the A/B/C lactam ring system **110** showed high regioselectivity in a Schmidt reaction to give lactam **111** in 55 % yield (Scheme 74).[98] Alkyl migration rather than aryl migration leads to the more stable cation, as the positive charge is stabilised at the benzylic position, which could be the reason for the formation of the desired regioisomer.



Scheme 74: Schmidt reaction to yield desired regioisomer **111** in 55 % yield[98]

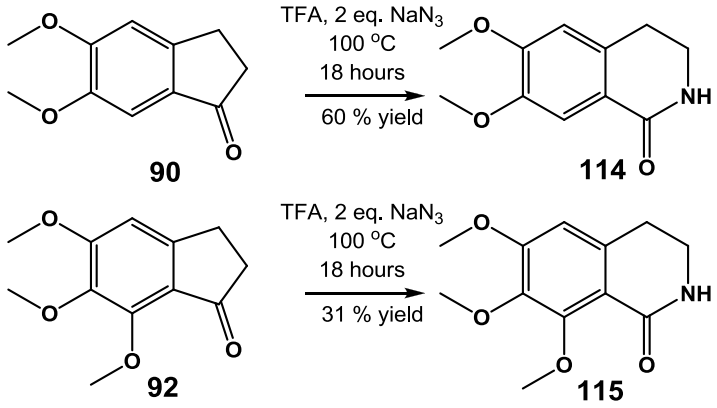
As previously discussed, Schmidt reactions attempted on A/B indanone **88** utilising previously reported conditions were unsuccessful. Instead of using PPA or H_2SO_4, reaction conditions of 2 eq. NaN_3 at 100 ^oC in TFA for 22 hours in a sealed pressure tube gave the best results (Scheme 69).

In 2006, a Beckmann rearrangment on a similar A/B/C indanone **112** was reported and the regioisomer **113** was isolated (Scheme 75).[99] No yield for this reaction was reported.



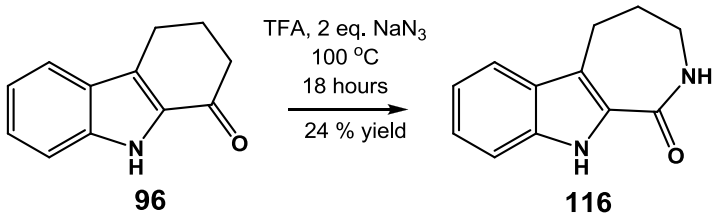
Scheme 75: Beckmann rearrangement using PPA to afford lactam **113** [99]

The optimised conditions using TFAA and 2 eq. NaN_3 at 100 ^oC in TFA for 22 hours in a sealed pressure tube developed to isolate lactam **101** (Scheme 69) have been applied to the other synthesised A/B indanones to give the desired corresponding lactams **114** and **115** from alkyl migration (Scheme 76). The other possible regioisomers were not observed in these reactions.



Scheme 76: Optimised Schmidt reactions to afford A/B lactams **114** and **115**

The desired regioisomer, lactam **116**, was also formed from a Schmidt reaction on cyclic ketone **96** in 24 % yield (Scheme 77).

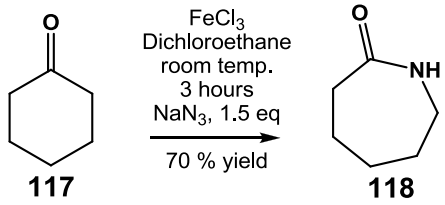


Scheme 77: Schmidt reaction on cyclic ketone **96** to afford lactam **116**

3.3. Tetrazoles

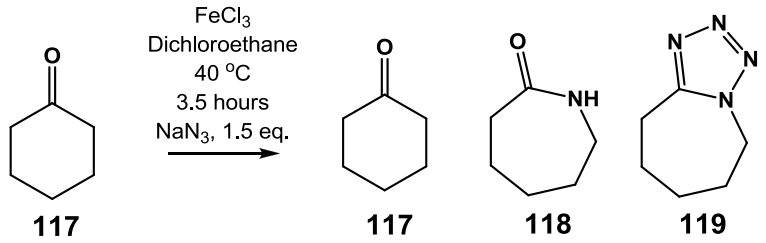
3.3.1. Optimisation

Yadav *et al.* recently reported a Schmidt reaction on a variety of substrates in the presence of $FeCl_3$, an example of which is shown in Scheme 78.[100] The identical procedure was repeated using cyclohexanone **117** but, unfortunately, no desired lactam **118** was formed and only starting material was recovered.



Scheme 78: Schmidt reaction in the presence of $FeCl_3$ to afford lactam **118** in 70 % yield[100]

Conducting the reaction at 40 °C for 3.5 hours led to partial reaction, shown in scheme 79. Crude ^1H NMR showed a mixture of starting material **117**, lactam **118** and also the unexpected tetrazole **119**.

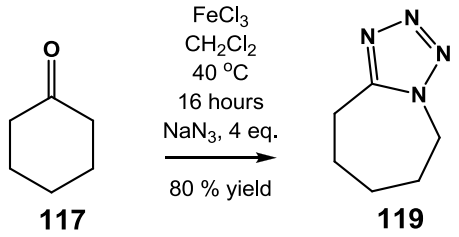


Scheme 79: Mixture of products from a Schmidt reaction on ketone **117**

Previously, this transformation of a cyclic ketone to its corresponding tetrazole had been reported using NaN_3 and $AlCl_3$.[101]

To prevent the formation of tetrazole **119**, the reaction was repeated using only 1 equivalent of NaN_3. This indeed did prevent the sythesis of the tetrazole and only lactam **118** was observed by ^1H NMR.

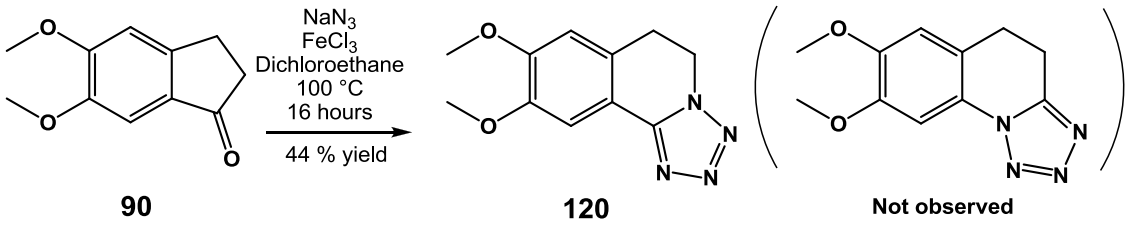
To investigate the formation of tetrazole **119**, the reaction was performed using 4 equivalents of NaN_3. The reaction was again carried out at 40 ^oC with $FeCl_3$ and CH_2Cl_2 as the solvent and the desired tetrazole **119** was isolated in 80 % yield (Scheme 80).



Scheme 80: Synthesis of tetrazole **117**

These optimised reaction conditions were then applied to the dimethoxy indanone **90** but the reaction did not give the desired product and only starting material was isolated. Dichloroethane was used as the solvent in order to cater for an increase in temperature to 84 ^oC. After 24 hours, only starting material was observed by TLC and after heating in a pressure tube at 120 ^oC for 6 hours, again only starting material was recovered.

Indanone **90** when subjected to $FeCl_3$, 4 equivalents of NaN_3 in dichloroethane at 100 ^oC in a pressure tube for 16 hours afforded tetrazole **120** in 44 % yield (Scheme 81). The regioisomer of the tetrazole was determined by HMBC NMR (see appendices **8.2.1.**).

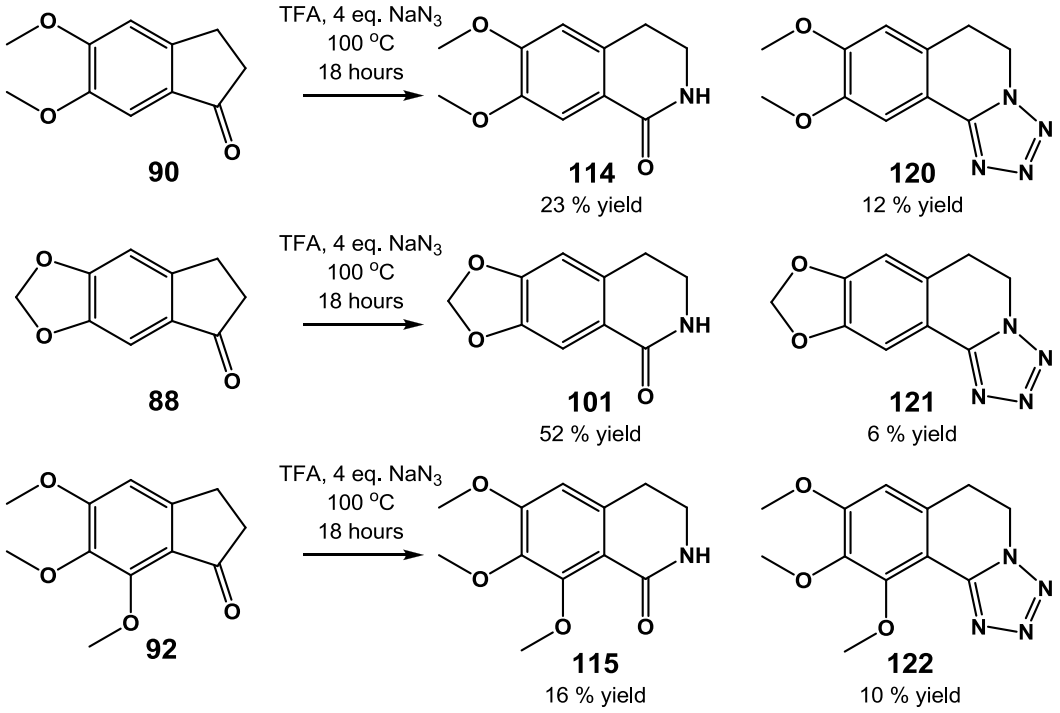


Scheme 81: Formation of tetrazole **120**

However, this reaction was extremely capricious and, when the exact conditions were repeated on the identical substrate **90**, only starting material was recovered.

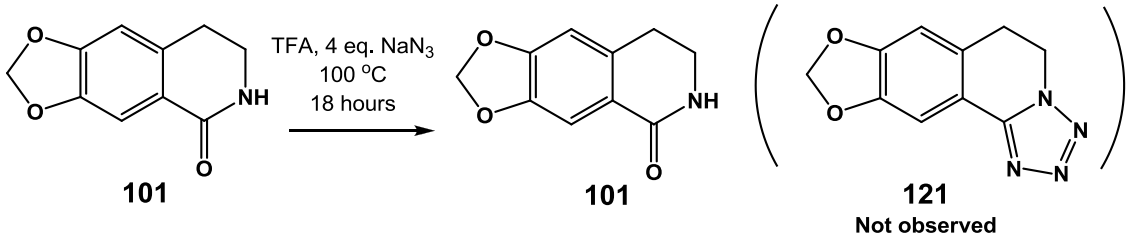
3.3.2. Synthesis and Mechanism

In the previous optimised Schmidt reactions (Scheme 76 and 77) only 2 equivalents of NaN_3 were used. The addition of 4 equivalents gave rise to not only the same desired lactams, though in diminished yields, but also their corresponding tetrazoles (Scheme 82). These reactions are low yielding, but are reproducible.



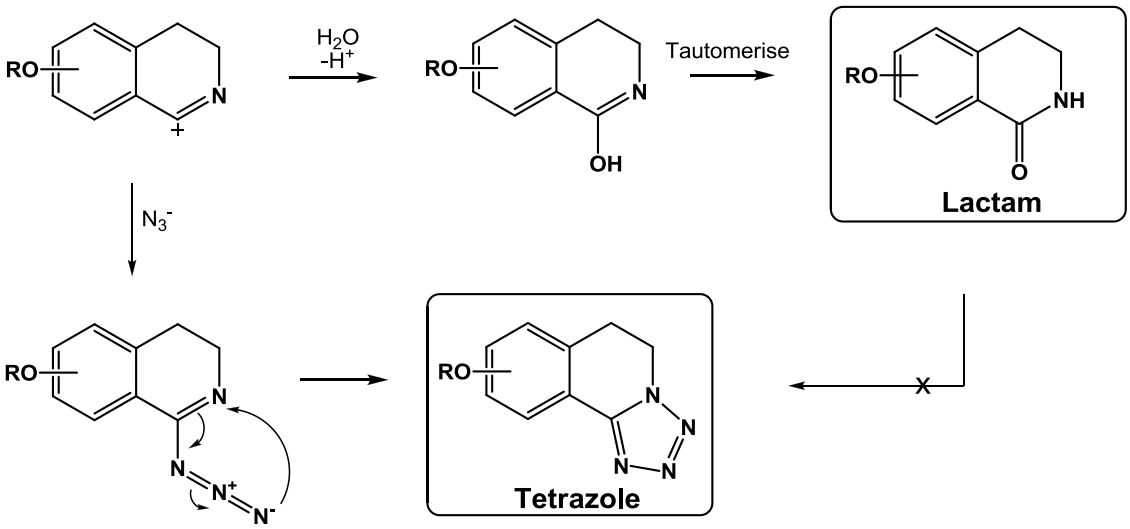
Scheme 82: Synthesis of tetrazoles **120**, **121** and **122**

The mechanism of this transformation was then investigated. As both the lactam and tetrazole are formed in the same reaction vessel, lactam **101** was placed back into the same reaction conditions, to determine whether any tetrazole is again formed, which would indicate the lactam is an intermediate towards the tetrazole. No tetrazole **121** was observed after 18 hours under the reaction conditions, only lactam remained, which suggests the lactam is not an intermediate towards the tetrazole (Scheme 83).



Scheme 83: Lactam **101** placed back into the same reaction conditions and no tetrazole **121** was observed

The proposed mechanism is outlined below (Scheme 84). The lactam is formed by capturing the benzylic cation by the addition of H_2O as the nucleophile. However, in the presence of an excess of NaN_3, the N_3^- nucleophile captures the cation instead which then cyclises to afford the tetrazole in the observed isomer.



Scheme 84: Proposed tetrazole mechanism

3.3.3. Biological Activity of Tetrazoles

Compounds containing the tetrazole moiety have been shown to possess a range of biological activities (Figure 20). Losartan is commonly used for the treatment of hypertension, TAK-456 is an antifungal agent and PTZ is used to treat anxiety.[102,103]

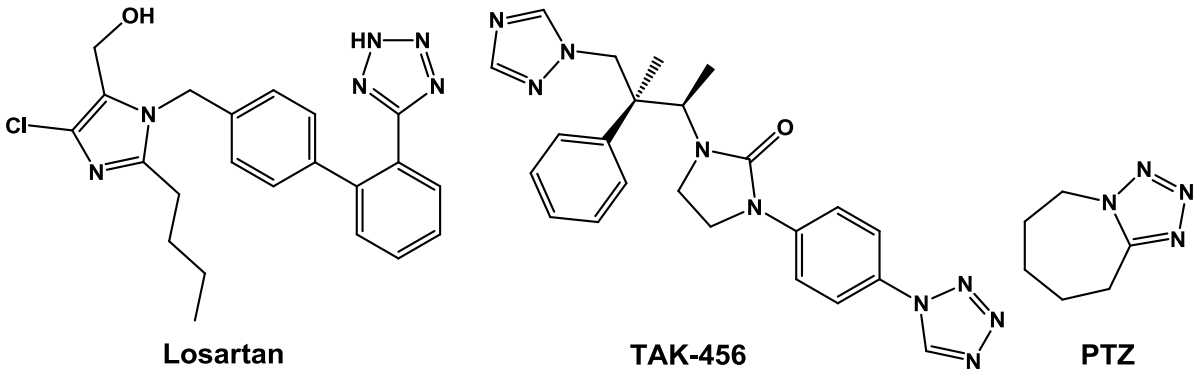


Figure 20: Drugs which contain the tetrazole moiety

Kumar *et al.* have reported the biological evaluation on tetrazole-containing compounds as potential anticancer agents.[103] In this report, a series of novel tetrazole containing analogues were synthesised and tested against a range of five cancer cell lines, showing promising anticancer activites. The three analogues below displayed good activity in the liver carcinoma cell line (HepG2) and also prostate cancer cell line (DU145) (Figure 21).

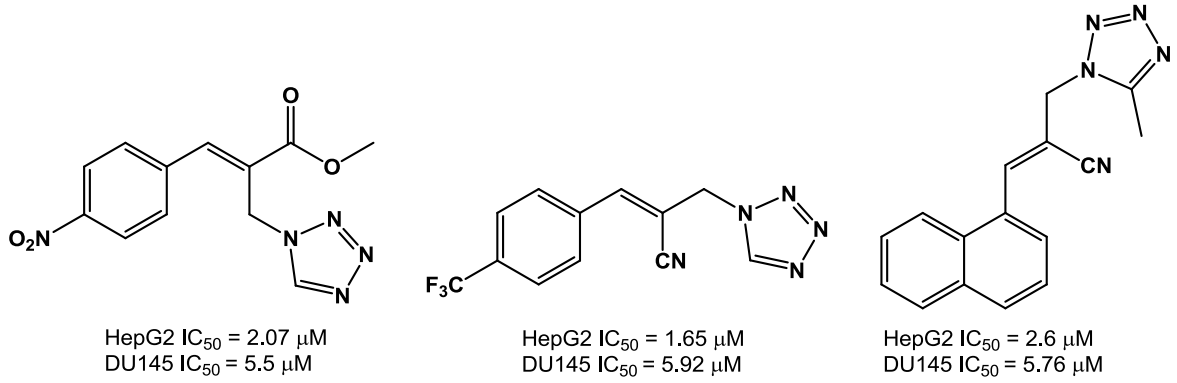


Figure 21: Three analogues containing the tetrazole moiety which show promising anticancer activity[103]

3.4. Oxidation Reactions

The 10b position on narciclasine **17** is sp^2 hybridised (Figure 22). To mimic this, oxidation of the A/B lactams has been investigated to determine whether this alters the biological activities of these simplified lactam analogues.

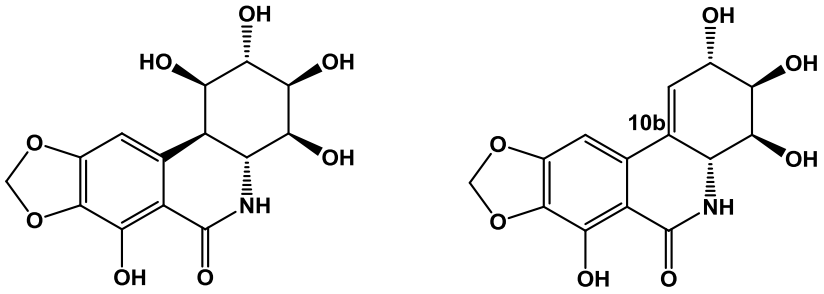
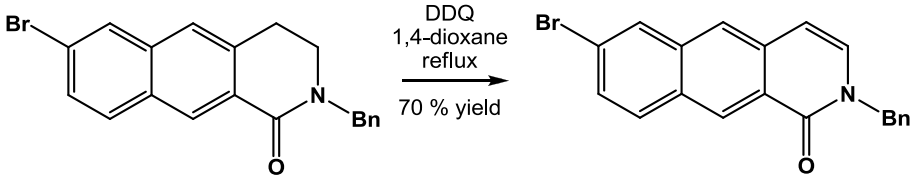


Figure 22: The sp^2 hybridisation at position 10b in narciclasine **17** enhances potency

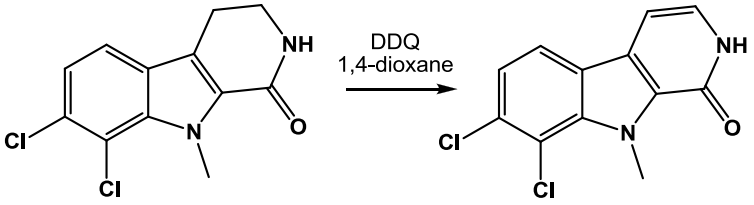
3.4.1. Dehydrogenation using DDQ

Tsai *et al.* reported the dehydrogenation of a lactam to afford its corresponding oxidised product in 70 % yield using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Scheme 85).[104]



Scheme 85: Dehydrogenation using DDQ in 1,4-dioxane under reflux[104]

Lingam *et al.* later also reported dehydrogenation using DDQ as a step in the first reported total synthesis of Bauerine C (Scheme 86).[105]



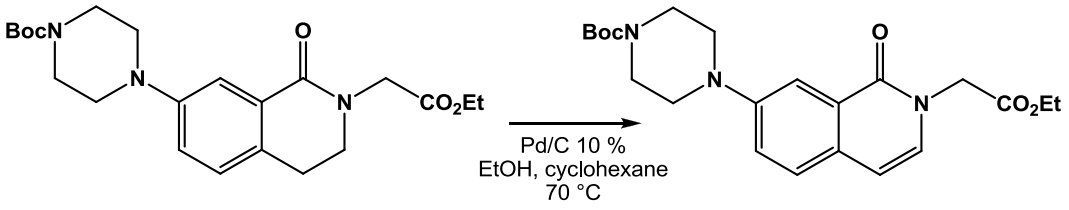
Scheme 86: Dehydrogenation in the synthesis of Bauerine C[105]

The reaction of DDQ (8 eq.) in 1,4-dioxane at 110 ^{o}C for 18 hours was performed on trimethoxy lactam **115**. Starting material and product were shown to be present by ^{1}H NMR spectroscopy of the crude reaction mixture. However, the DDQ and its products proved extremely difficult to remove from the desired material.

Previous work within the group involved similar transformations using DDQ as a reagent, these reactions also obtained low yields as the products were difficult to isolate and therefore an alternative procedure was investigated.

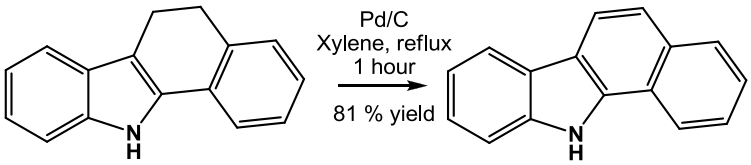
3.4.2. Dehydrogenation using Pd/C

Hutchinson *et al.* reported the successful dehydrogenation of a cyclic lactam to afford its oxidised product, using Pd/C in EtOH (Scheme 87).[106]



Scheme 87: The oxidation of a lactam using Pd/C[106]

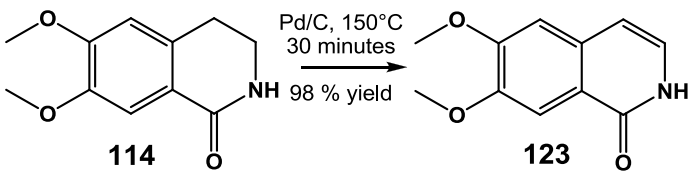
Later, Dufour and Kirsch also reported oxidation by use of Pd/C in xylene (Scheme 88).[107]



Scheme 88: Oxidation of a lactam using Pd/C in xylene[107]

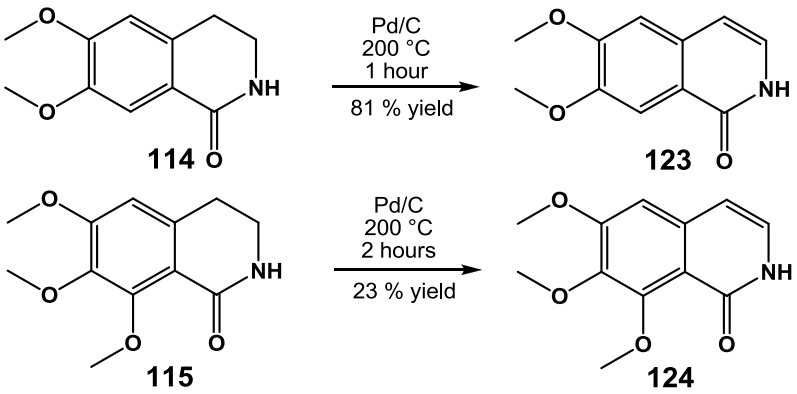
Following this procedure reported by Dufour and Kirsch, trimethoxy lactam **113** and Pd/C in xylene were heated at reflux for 24 hours. However, only starting material was recovered.

Awvah and Capretta reported this transformation using solvent-free conditions on dimethoxy lactam **114** to isolate desired product **123** in 98 % yield (Scheme 89).[108]



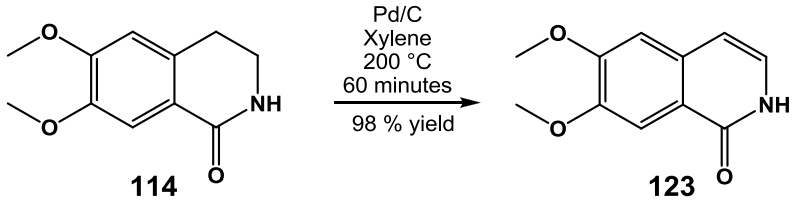
Scheme 89: Oxidation of lactam **114** in solvent-free conditions using Pd/C at 150 ^{o}C for 30 minutes

These conditions were repeated on the identical substrate **114**, unfortunately only starting material was recovered. After discussions with the authors, they suggested using previously reported solvent-free conditions at 200 - 210 ^{o}C.[109] These conditions were repeated under an argon atmosphere as described and the desired products **123** and **124** were isolated (Scheme 90).



Scheme 90: Oxidations of A/B lactams **114** and **115** to afford compounds **123** and **124**

The large difference in yields of these two substrates demonstrates that these are very capricious reactions. An alternative method which was explored is the use of a microwave. Lactam **114** and Pd/C in xylene at 200 ^{o}C in a microwave for 60 minutes afforded lactam **123** in 98 % yield (Scheme 91). This reaction is reproducible and these appear to be the most efficient conditions for this transformation.



Scheme 91: Successful oxidation of lactam **114** in the microwave to afford compound **123** in 98 % yield

3.5. Biological Activity

The A/B lactam and tetrazoles have been tested in an MTS cell proliferation assay using colon cancer cell line HT29 and breast cancer cell line MDA-MB-231.

Previously synthesised *via* a different route and tested on the HT29 cell line within the group were the A/B lactams and A/B oxidised lactams (Figure 23).[110]

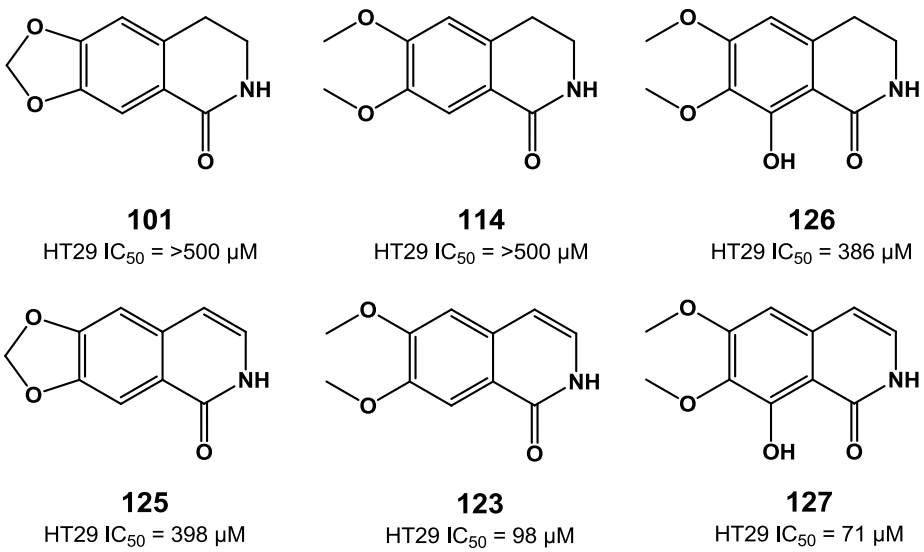


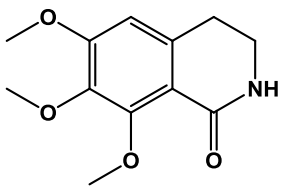
Figure 23: Compounds previously synthesised and tested within the group[110]

The weak activities of these A/B lactam analogues are expected. This is because they all lack the C-ring which, as explained previously in the structure-activity relationship of pancratistatin, is required to retain the activity (**1.2.3.3.**). As previously discussed, the presence of the free phenol in pancratistain has been shown to increase the activity by 10-fold. Lactam **126** would therefore be expected to display increased activity compared to lactam **114**, although the observed difference is not significant.

Oxidised products (**125**, **123** and **127**) show improved activity, which mimics the difference between pancratistatin and narciclasine. Pancratistatin **16** and narciclasine **17**, when tested in the National Cancer Institute 60 cell line screen, displayed GI_{50} values of 0.091 μM and 0.016 μM, respectively. Narciclasine **17** contains the sp^2-hybridised 10b position and its activity is slightly greater than pancratistatin **16**. The oxidised A/B lactams also demonstrate this improved activity compared to their corresponding hydrogenated lactam analogues (Figure 23).

Trimethoxy lactam **115** was tested in both HT29 and MDA231 cancer cell lines (Figure 24, see appendices **8.2.2.**). The IC_{50} value of lactam **115** was above the highest measured

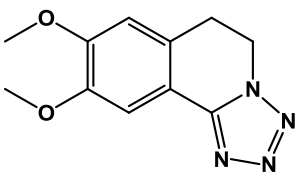
concentration of 500 μM. Methylenedioxy lactam **101** and dimethoxy lactam **114** also gave an IC_{50} value >500 μM.



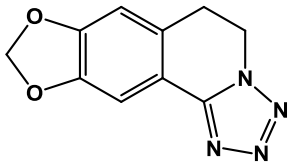
115
HT29 IC_{50} = 448 μM
MDA231 IC_{50} = >500 μM

Figure 24: IC_{50} values of trimethoxy lactam **115** in HT29 and MDA231 cancer cell lines

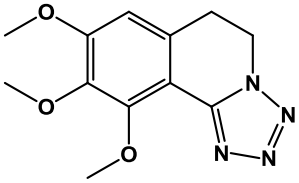
Due to the potential activity of the tetrazole side products, the analogues synthesised were also tested in both HT29 and MDA231 cancer cell lines (Figure 25, see appendices **8.2.2.**). These compounds were not active in either of the colon or breast cancer cell lines, so were not pursued any further. Oxidation of these compounds was considered but the stability of these tetrazoles at such high temperatures is unclear, so oxidations were not performed.



120
HT29 IC_{50} = >500 μM
MDA231 IC_{50} = >500 μM



121
HT29 IC_{50} = >500 μM
MDA231 IC_{50} = >500 μM



122
HT29 IC_{50} = >500 μM
MDA231 IC_{50} = >500 μM

Figure 25: IC_{50} values of tetrazoles **120**, **121** and **122** in HT29 and MDA231 cancer cell lines

3.6. Conclusions

The aim of the work described within this chapter was to synthesise a range of A/B indanones that could subsequently be transformed into their corresponding lactams. Indanone formation was conducted using a range of commercially available substituted propanoic acids. The formation of indanones was acheived *via* an intramolecular Friedel-Crafts acylation reaction using TFAA as an activator and a library of indanones were synthesised in high yield.

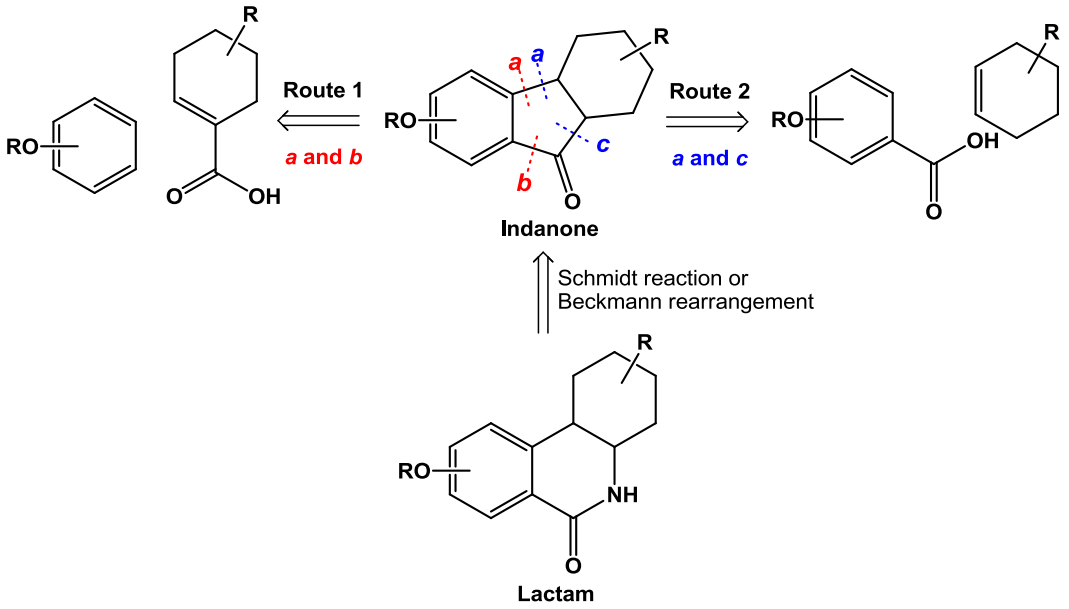
A Schmidt reaction or Beckmann rearrangement was proposed to access the A/B lactam framework from the indanone intermediate. Schmidt reactions were first attempted for a range of indanones and proved successful; due to the success of these transformations Beckmann rearrangements were not attempted. The Schmidt reaction conditions were then optimised and were used to synthesise a library of A/B lactams from their corresponding indanones in good yield. During the optimisation of the Schmidt reaction conditions the synthesis of tetrazoles was observed when a large excess of NaN_3 was used. This phenomenon was explored further and a range of tetrazoles were synthesised in low yield.

The lactams and tetrazoles were submitted for biological testing for growth inhibition against HT29 and MDA231 cancer cell lines. IC_{50} values were not observed for any of the tetrazoles, with concentrations tested up to 500 µM. A range of lactams were also tested and showed low activity, with IC_{50} values ranging from 71 µM to >500 µM. No significant activity was anticipated for these lactams as they lack a C-ring and associated functionality.

4. Chapter Four - A/B/C Analogues of Dihydroisoquinolinones

4.1. Proposed Synthetic Route

The proposed synthetic route can also be applied to the A/B/C ring system through two main transformations. The A/B/C lactam framework will also be accessed *via* an indanone intermediate using a Schmidt reaction or Beckmann rearrangement. The indanone intermediate could potentially be synthesised through two different routes, depending on the disconnection approach used (Scheme 92).



Scheme 92: Proposed disconnection approach to synthesise A/B/C ring system through two main transformations

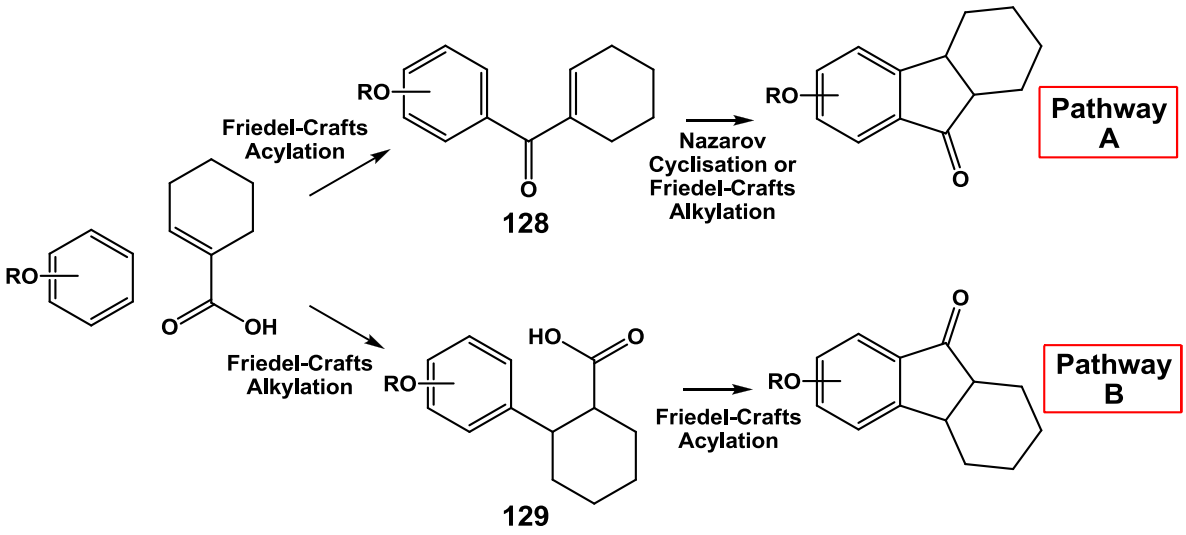
Disconnections at positions ***a* and *b*** results in commercially available starting materials α,β-unsaturated cyclohexene carboxylic acid and a functionalised benzene ring (**Route 1**). The second approach uses alternative disconnections at positions ***a* and *c***. The two commercially available starting materials comprise of a functionalised cyclohexene ring and a substituted benzoic acid (**Route 2**).

Once optimised this synthetic strategy of utilising two different routes towards the indanone intermediate enables flexibility in the variety of commercially available starting materials which could potentially be used. This facilitates the ability to produce a wider range of novel non-natural analogues of the pancratistatin and narciclasine core.

As previously discussed the second transformation to form the lactam scaffold from its corresponding indanone can be performed using NaN_3 under acidic conditions to undergo a Schmidt reaction. Alternative conditions to afford the lactam core utilises a Beckmann rearrangement which is performed with NH_2OH under acidic conditions.

4.1.1. Route 1 - Intermolecular Indanone Synthesis - Mechanistic Pathways

Indanone synthesis following Route 1 with disconnections ***a*** **and** ***b*** (Scheme 92) provides an α,β-unsaturated cyclohexene carboxylic acid and a functionalised benzene ring as starting materials. This reaction mechanistically can proceed through two different pathways (Scheme 93).



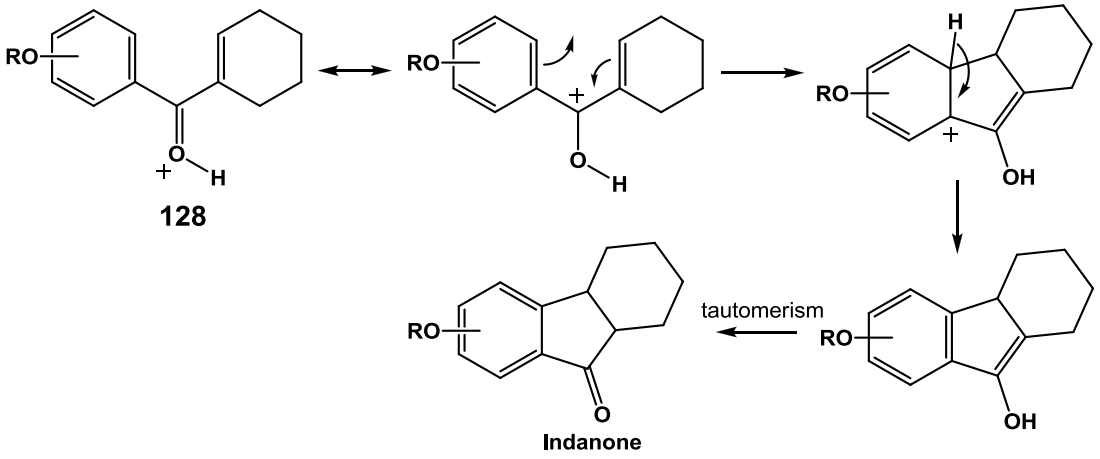
Scheme 93: Indanone synthesis *via* Route 1 which can follow two different mechanistic pathways

If the α,β-unsaturated carboxylic acid and benzene ring starting materials initially undergo a Friedel-Crafts acylation reaction to form the aryl vinyl ketone **128**. This intermediate can then undergo either a Nazarov cyclisation or an intramolecular Friedel-Crafts alkylation to afford the desired indanone (**Pathway A**).

Alternatively, the α,β-unsaturated carboxylic acid and benzene ring in the initial step could react through a 1,4 conjugate addition to afford acid intermediate **129**, followed by an intramolecular Friedel-Crafts acylation reaction (**Pathway B**) to yield the same indanone.

4.1.2. Route 1 - Nazarov Cyclisation

Divinyl ketone **128** intermediate formed in Pathway A can undergo a Nazarov cyclisation, a ring-closing 4π electrocyclisation reaction, to afford the desired indanone.[111] For this transformation to occur, acidic reaction conditions are required to protonate the ketone, which forms a favourable conjugated π system, five p-orbitals with 4π electrons, to encourage the electrocyclisation to proceed (Scheme 94).



Scheme 94: Nazarov cyclisation mechanism

In 2006, Liang *et al.* reported the synthesis of similar structures Taiwaniaquinoids (Figure 18) using a Nazarov triflation procedure.[112]

4.1.3. Route 1 - Introducing Functionalisation

Route 1 must be capable of incorporating substitution both on the A-ring and the C-ring in order to achieve the functionalisation required to retain activity.

There are a range of commercially available substituted benzene rings, including anisole, 1,3-benzodioxole and 1,2,3-trimethoxybenzene (Figure 26). An example of a more functionalised α,β-unsaturated carboxylic acid is commercially available shikimic acid (Figure 26). This

may require a protection-deprotection strategy in order to obtain the highly oxygenated C-ring of pancratistatin and narciclasine analogues.

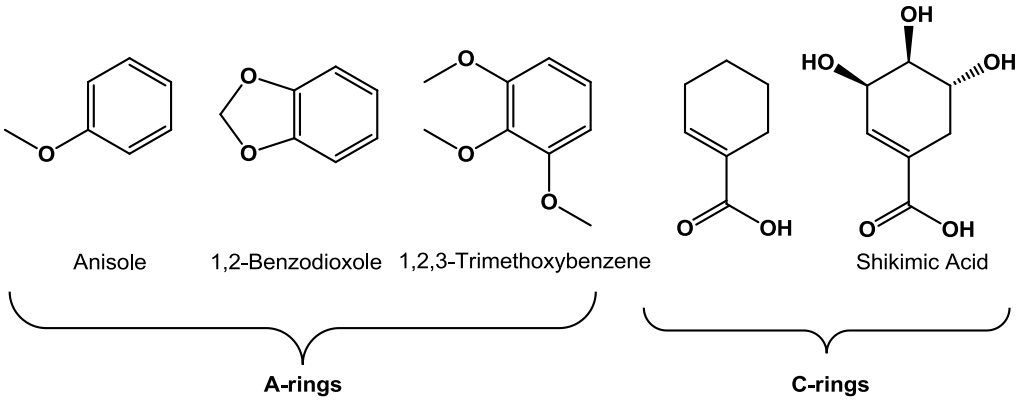
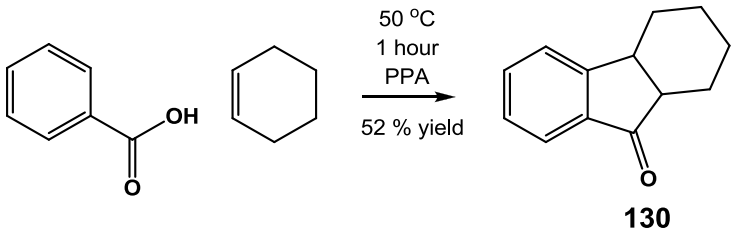


Figure 26: Commercially available starting materials to incorporate substitution *via* Route 1

4.1.4. Route 2 - Intermolecular Indanone Synthesis

Complementary Route 2 follows two different disconnections at positions ***a* and *c*** (Scheme 92). The commercially available starting materials include a cyclohexene ring and a substituted benzoic acid.

An example of this transformation was previously reported by Rand and Dolinsky between benzoic acid and cyclohexene in polyphosphoric acid (PPA) to afford indanone **130** in 52 % yield (Scheme 95).[113]



Scheme 95: Synthesis of Indanone **130** in the presence of PPA[113]

4.1.5. Route 2 - Introducing Functionalisation

Incorporation of substitution on the A-ring and C-ring is also important in Route 2. A variety of commercially available substituted olefins could be used to gain interesting substitution around the C-ring. Also various substituted benzoic acids are commercially available to

achieve functionalisation on the A-ring (Figure 27). The regioselectivity in the indanone formation of the functionalised cyclohexene C-rings will depend on the type of olefin used.

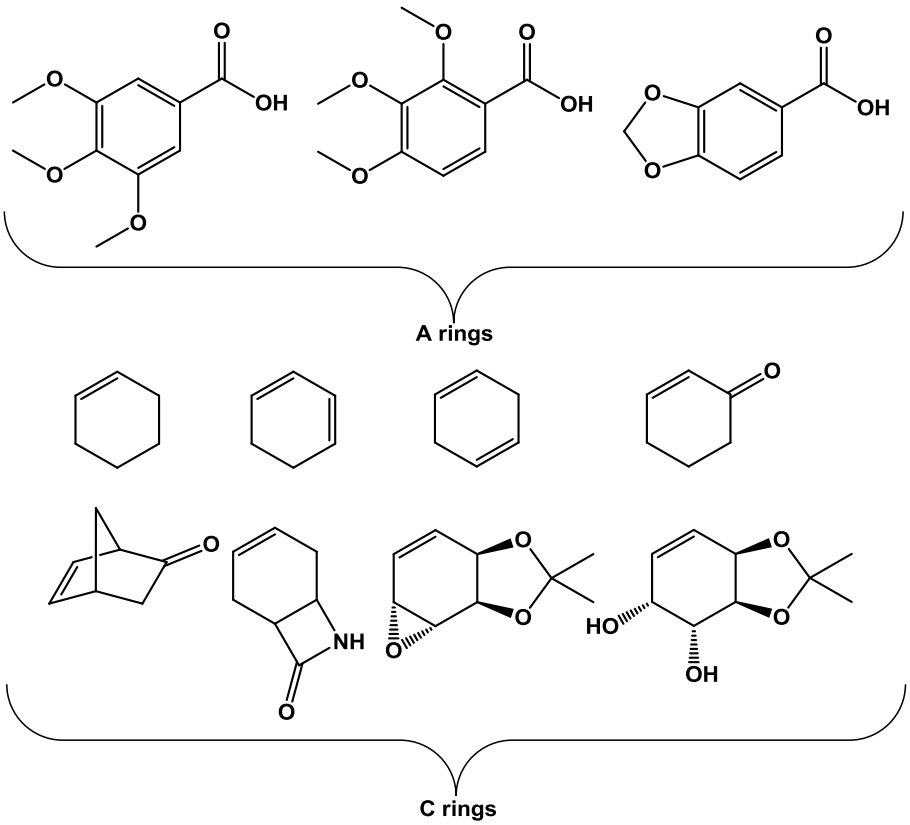
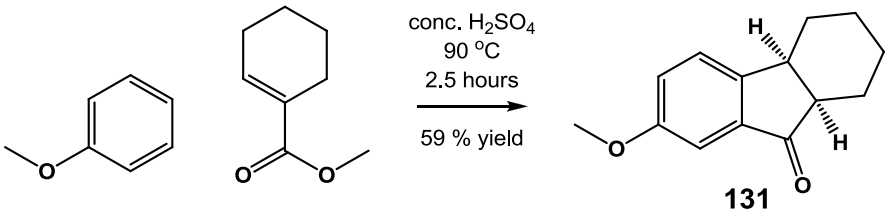


Figure 27: Commercially available starting materials to incorporate functionalisation *via* Route 2

4.2. Results and Discussion

4.2.1. Route 1 - Intermolecular Indanone Synthesis

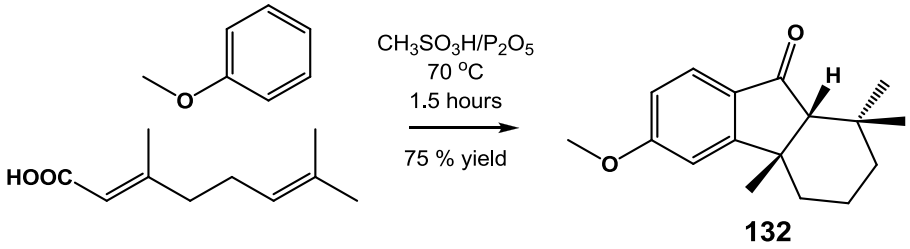
Ramana *et al.* synthesised thermodynamically more stable *cis*[114] indanone **131** in 59 % yield from anisole and an α,β-unsaturated ester in concentrated H_2SO_4 at 90 ^{o}C (Scheme 96).[81] The methoxy group present in anisole is electron-donating and therefore directs *ortho* or *para*. The indanone **131** obtained shows that this mechanism proceeded initially with a 1,4-addition followed by a Friedel-Crafts acylation reaction following Pathway B (Scheme 93).



Scheme 96: Synthesis of *cis* indanone **131**

This experimental procedure using the same reagents, anisole and α,β-unsaturated methyl ester, was performed to check the reproducibility of this literature example. However, mostly starting materials were present by crude ^{1}H NMR. Only when the crude sample was submitted to mass spectrometry was a peak corresponding to the product identified.

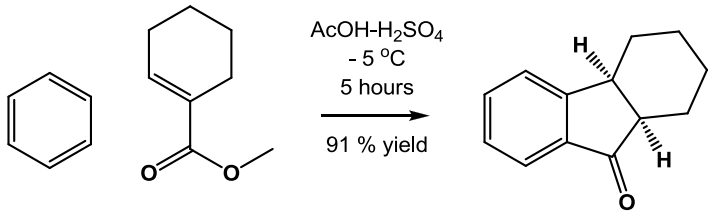
Tang *et al.* developed an efficient Friedel-Crafts acylation followed by a Friedel-Crafts alkylation to afford the skeleton of the taiwaniaquinoids[5] following Pathway A. In this report reactions were attempted on reagents anisole and geranic acid (Scheme 97). Various acidic media were tried including H_2SO_4 with AcOH, PPA, $BF_3{\cdot}Et_2O$ and methanesulfonic acid with phosphorus pentoxide. The most successful reaction reported was methanesulfonic acid with phosphorus pentoxide which yielded 75 % of the desired indanone **132**.



Scheme 97: Synthesis of indanone **132** in 75 % yield[5]

The identical reaction was performed as reported; however the TLC and ^{1}H NMR spectrum of the crude reaction mixture showed complicated mixtures of inseparable compounds. The reaction was repeated under a N_2 atmosphere and also at room temperature though these reactions were again unsuccessful. The same reaction conditions were attempted using anisole with cyclohexene-1-carboxylic acid, from which only a trace of impure indanone was obtained after column chromatograhy.

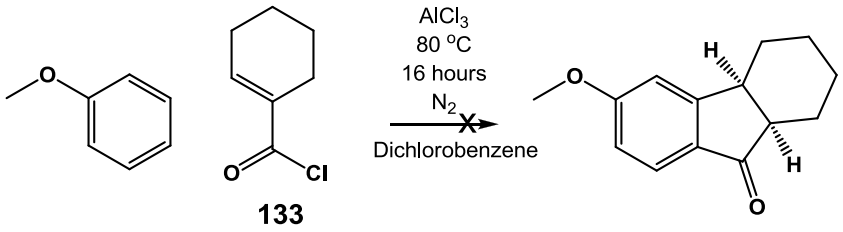
Successful indanone syntheses using substituted benzene rings with α,β-unsaturated methyl esters in the presence of AcOH and H_2SO_4 (1: 9) at –5 ^oC have been reported (Scheme 98).[115] The report states their mechanism proceeds through a conjugate addition followed by a cyclo-acylation reaction (Pathway B). When the reaction was carried out, no desired product was obtained; instead the starting material was isolated in 75 % yield.



Scheme 98: Reported indanone synthesis using AcOH-H_2SO_4[115]

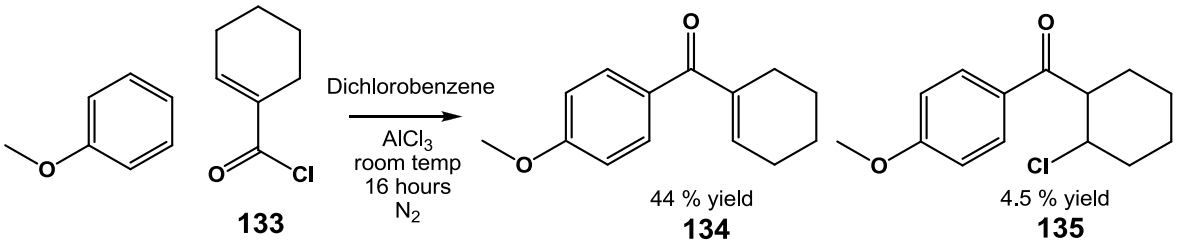
The syntheses of a series of indanones were reported using α,β-unsaturated acyl chlorides and $AlCl_3$ in dry dichlorobenzene under microwave conditions.[116] The reported mechanism proceeds *via* a tandem Friedel-Crafts acylation followed by a Nazarov cyclisation (Pathway A).

Acid chloride **133** was synthesised from its carboxylic acid using either thionyl chloride[117] or oxalyl chloride[118]. Similar conditions using $AlCl_3$, under N_2 at 80 ^oC were attempted using cyclohexene-1-carbonyl chloride **133** and anisole (Scheme 99). Unfortunately the starting materials decomposed and no desired indanone or starting material was isolated.



Scheme 99: Unsuccessful indanone formation from acid chloride **133** and $AlCl_3$

The same reaction conditions were then repeated at room temperature and a mixture of products were obtained. After column chromatography, uncyclised alkene **134** was isolated in 44 % yield and chloride **135** was isolated in 4.5 % yield (Scheme 100).



Scheme 100: Synthesis of compounds **134** and **135** at room temperature in the presence of $AlCl_3$

Under these reaction conditions, the methoxy group present in anisole demethylated and reacted to afford a mixture of other products which were identified by HRMS but not isolated (Figure 28).

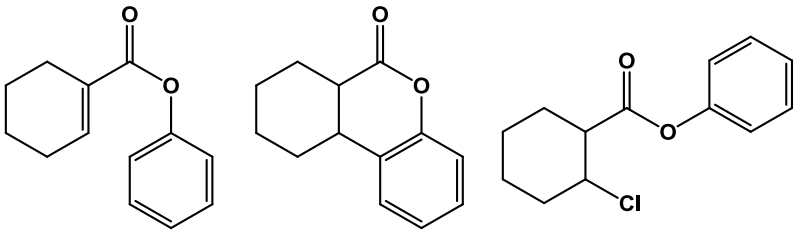


Figure 28: By-products from reaction shown in Scheme 100

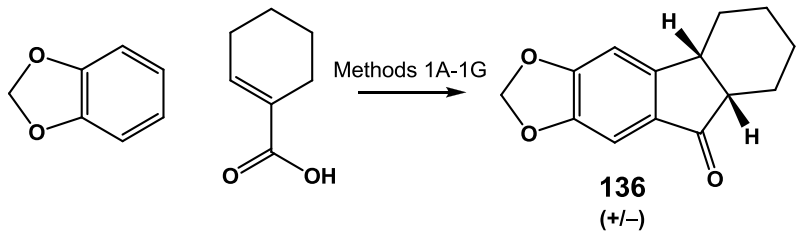
The isolation of alkene **134** reinforces the reported mechanism by Yin *et al.* of an initial Friedel-Crafts acylation reaction followed by the Nazarov cyclisation or Friedel-Crafts alkylation using $AlCl_3$ (Pathway A).[116]

A similar reaction of cyclohexenoyl chloride **133** and $AlCl_3$ in dry benzene, under reflux for five hours has been reported to obtain the corresponding indanone in 65 % yield.[119] When these exact reaction conditions on identical substrates were repeated, only starting material was recovered.

Nicolaou *et al.* reported the use of $BF_3{\cdot}Et_2O$ to perform a Friedel-Crafts acylation reaction.[80] A variety of conditions and substrates were attempted at temperatures ranging from room temperature to 120 ^oC and no indanones were recovered.

Due to the previous success of TFAA on the A/B intramolecular indanone analogues, this was also applied to the intermolecular indanone synthesis. Reactions with anisole or at lower

temperatures of 50 ^{o}C gave no desired indanone. Therefore, optimisation was carried out on 1,3-benzodioxole and cyclohexene-1-carboxylic acid (Scheme 101).



Scheme 101: Optimisation of indanone **136** *via* Route 1

Different ratios of TFA: TFAA were used, the work-up was modified, the equivalents of benzodioxole were varied and the time of reaction was also adjusted (Table 5). Optimum conditions (Method 1I) of cyclohexene-1-carboxylic acid and 2 eq. of 1,2-benzodioxole in TFAA at 100 ^{o}C for 4 hours afforded desired indanone **136** in 84 % yield. The addition of other acids were also investigated; however, the yields did not improve.[120] The same reaction was also attempted using methyl cyclohexene-1-carboxylate but only starting material was recovered, indicating the starting material must be as the free acid for this reaction to proceed.

Method	**TFAA:TFA ratio**	**Aq. wash**	**Addition of acid?**	**Eq. of Benzodioxole**	**Time (h)**	**Yield of 136**
1A	1:2	No	No	1.0	1	66%
1B	1:2	No	No	1.0	3.5	82%
1C	1:2	No	No	1.0	6	72%
1D	1:2	No	No	1.1	17	56%
1E	1:2	Yes	No	2.0	4	59%
1F	1:0	Yes	No	2.0	4	75%
1G	1:0	Yes	H_3PO_4	2.0	4	57%
1H	1:0	Yes	H_2SO_4	2.0	4	31%
1I	**1:0**	**No**	**No**	**2.0**	**4**	**84%**

Table 5: Optimisation of the reaction conditions to afford indanone **136** using 1 eq. of cyclohexene-1-carboxylic acid at 100 ^{0}C

As previously discussed (**1.2.3.3.**) the indanone must have a *trans* fused B/C ring junction to retain biological activity of these analogues. The dihedral angle of the two hydrogen atoms was modelled using Chem 3D Pro 11 and MM2 minimisation. The angles were calculated at ~36^{o} for the *cis* geometry and ~167^{o} for the *trans* geometry. Using the Karplus equation[121] the *J* coupling between the two hydrogen atoms was predicted to be ~7.5 Hz for the *cis* and ~12 Hz for the *trans* isomer. The *J* coupling between the two hydrogen atoms was observed as 7.0 Hz by ^{1}H NMR spectroscopy, indicating the product is the *cis* isomer. The relative configuration was confirmed by an X-ray crystal structure (Figure 29). The cystal structure shows that the H(5) and H(10) hydrogen atoms reside as the *cis* isomer relative to each other, with a H(5)-C(5)-C(10) angle of 110.7(1) ^{o} and a C(5)-C(10)-H(10) angle of 106.3(1) ^{o}. Also, the C(11)-O(3) bond length is 1.2236(15) Å, which is consistent with a C=O double bond.

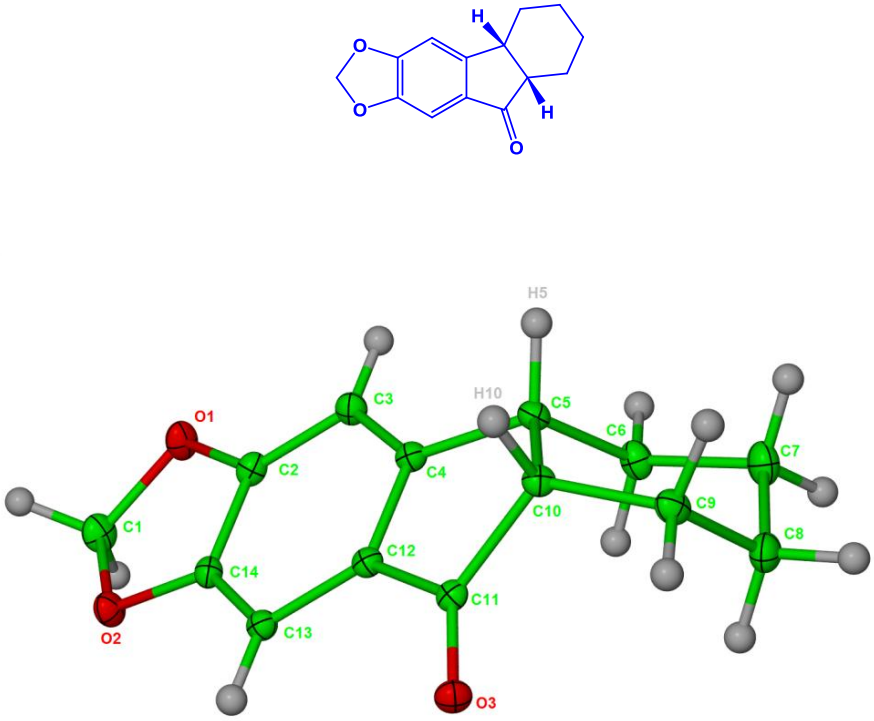


Figure 29: Crystal structure of indanone **136**

House and Carlson have shown that, for this type of compound, the *cis* isomer is more stable.[114] The *cis* geometry is not ideal in the synthesis of pancratistatin analogues but, once

substitution is introduced to the model compounds, the most stable isomer may be the desired *trans* geometry.

Some natural lycorine alkaloids, which are also isolated from the *Amaryllidaceae* species of plants, contain a *cis* B/C ring junction and also possess a wide range of biological activities (Figure 30).[122]

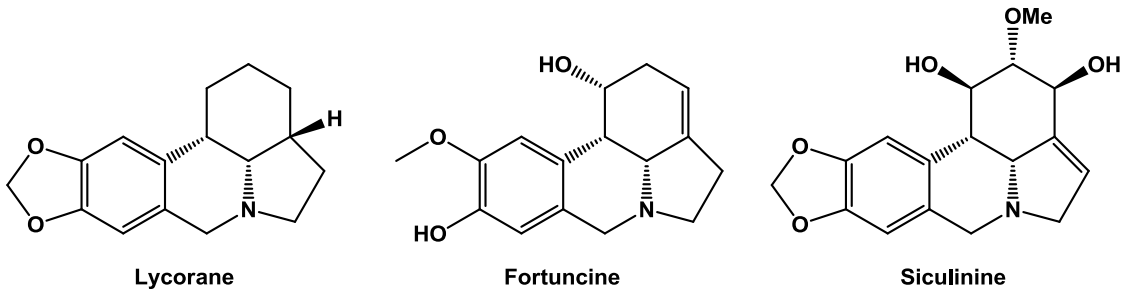
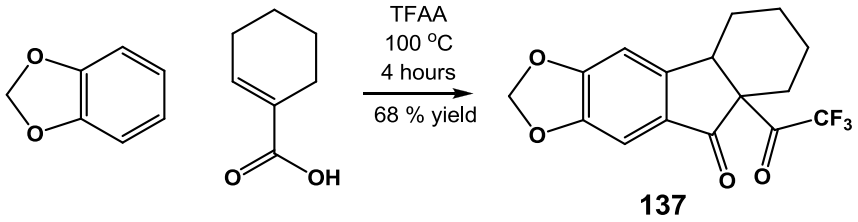


Figure 30: Lycorine-type alkaloids with *cis* B/C fused rings

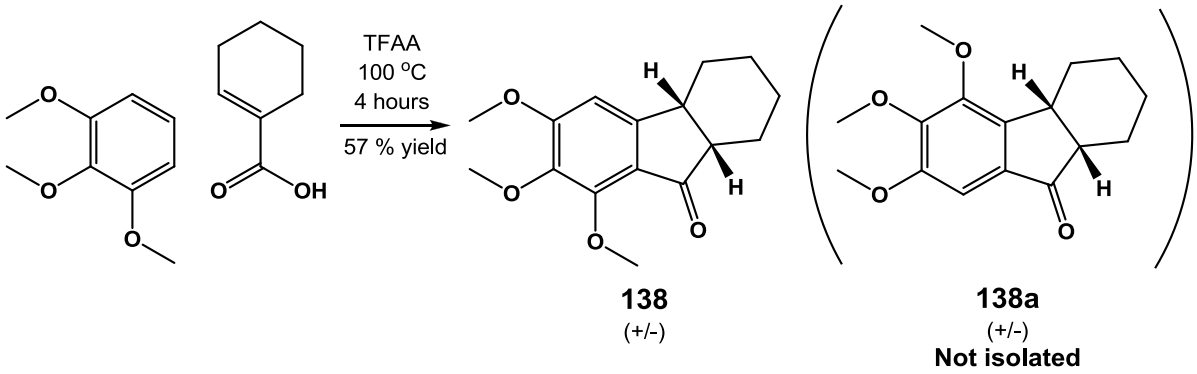
Optimised conditions (Method 1I) were repeated using the same substrates to ensure its reproducibility. The yields of indanone **136** in the reactions were fairly consistent, which implies that the reaction is reliable. However, on one occasion, another unexpected product was isolated after column chromatography, indanone **137** (Scheme 102). This was not a stable compound and decomposed over time. This is an unlikely compound to survive after column chromatography and, after many other attempts, this alternative product was not isolated or observed again.



Scheme 102: Unexpected observed indanone **137**

The use of 1,3-benzodioxole as a starting material does not provide any information on the mechanism of this reaction, owing to its symmetry. Therefore, the use of 1,2,3-trimethoxybenzene as the nucleophile was attempted and indanone **138** was obtained in 28 % yield. A repeat of this same reaction with aqueous work-up gave an improved 57 % yield of

indanone **138** (Scheme 103). The regioselectivity was determined by NOESY NMR, where irradiation of Ha gave NOE to one methoxy and three aliphatic protons (Figure 31). Regioisomer **138a** was not observed in either reaction which indicates that the mechanism most likely goes *via* an initial Friedel-Crafts acylation, followed by a Nazarov cyclisation (Pathway A). However, the failure to observe the formation of **138a** is not conclusive evidence of the order of the two steps. The conjugate addition could have taken place first at the 5-position of the trimethoxybenzene, followed by ring-closing Friedel-Crafts reaction (Pathway B). This reaction was very capricious and it was not always possible to access the desired indanone in as good yield.



Scheme 103: Formation of trimethoxyindanone **138** in 57 % yield

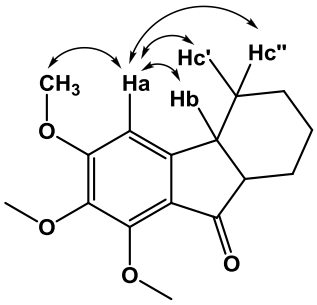
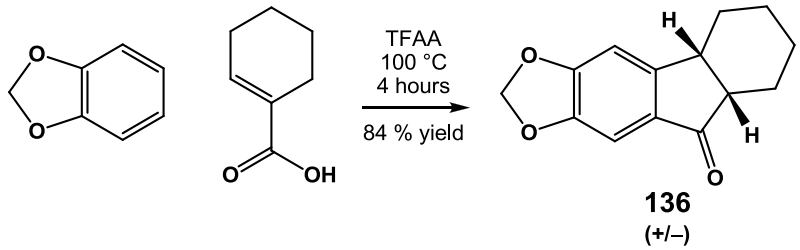


Figure 31: NOESY connectivities of protons on indanone **138**

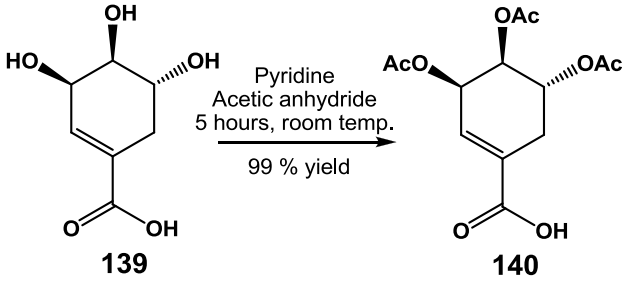
4.2.2. Route 1 - Introducing Functionality

Optimised conditions using 1,3-benzodioxole and cyclohexene-1-carboxylic acid to afford indanone **136** in 84 % yield were applied to other α,β-unsaturated carboxylic acids to yield the desired corresponding indanones (Scheme 104).



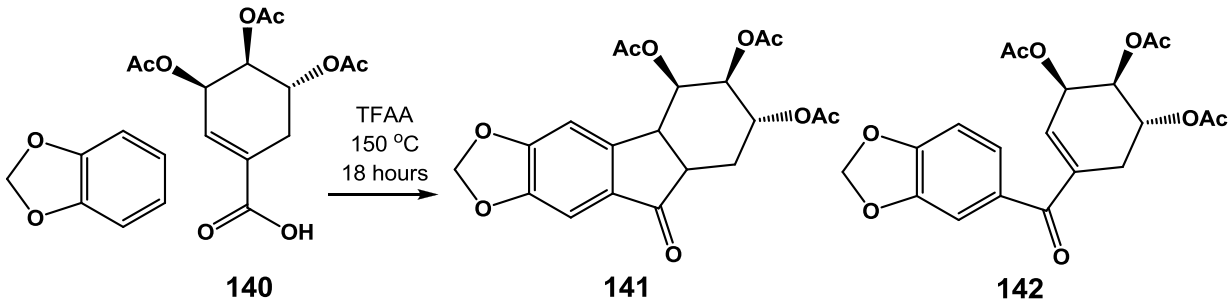
Scheme 104: Optimised conditions *via* Route 1

Commercially available shikimic acid **139** was protected as the free acid in a one step reaction, forming the triacetate **140** in quantitative yield, following literature precedent (Scheme 105).[123]



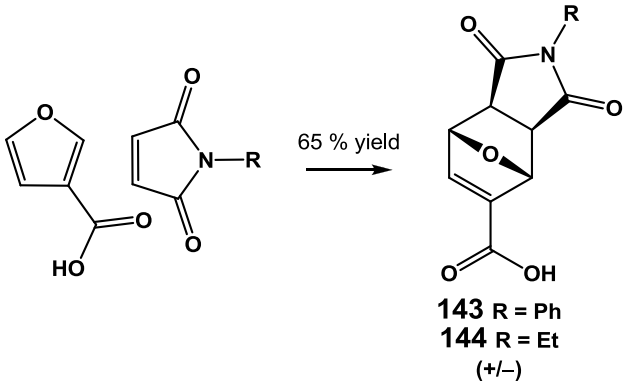
Scheme 105: Protection of shikimic acid with acetate esters **140**[123]

A variety of reactions were attempted at room temperature, 50 ^{o}C and 100 ^{o}C but unfortunately these were all ineffective at yielding the desired indanone **141**. The reaction was then carried out at 150 ^{o}C for 18 hours. After attempts at purification, trace amounts of products corresponding to **141** and **142** were observed in both ^{1}H NMR and HRMS (Scheme 106, see appendices **8.3.2.** for ^{1}H NMR and HRMS of desired compound **141**). The observation of a signal corresponding to **142** in the ^{1}H NMR spectrum of the crude reaction mixture reinforces the proposal that this mechanism procedes *via* an initial Friedel-Crafts acylation reaction, followed by a Nazarov cyclisation.



Scheme 106: Synthesis of indanone **141** using protected shikimic acid

Due to the protection, deprotection strategy required for shikimic acid, alternative C-rings were also investigated. Following the procedure reported by Ravikumar *et al.,* 3-furoic acid and *N*-phenylmaleimide were heated at reflux in MeCN to yield the Diels-Alder product **143** in 65 % yield.[124] *N*-Ethylmaleimide and 3-furoic acid in MeCN at reflux afforded the Diels-Alder product **144** also in 65 % yield (Scheme 107). Only the *exo* products were observed in both these reactions and no *endo* products were isolated, which was established by utilising the Karplus equation.



Scheme 107: Observed *exo* products from Diels-Alder reaction

The dihedral angle H^A-C-C-H^B for the *endo* product is 35.4° and 82.2° for the *exo* (Figure 32). Using the Karplus equation, [121] the vicinal ^3J coupling constants will vary depending on whether the product is *endo* or *exo*. The *endo* product with a dihedral angle of 35.4° will have a ^3J coupling of ~10 Hz and the *exo* product with a dihedral angle of 82.2° will have an extremely low ^3J coupling, if any is seen. No coupling is observed by ^{1}H NMR between these two protons in products **143** and **144**, which is consistent with the *exo* product.

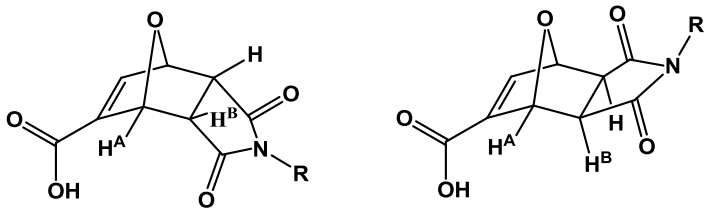
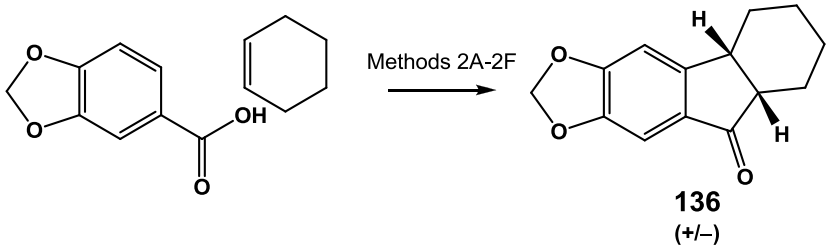


Figure 32: *Endo* and *exo* products

The α,β-unsaturated carboxylic acids **143** and **144** were subjected to the optimised intermolecular indanone synthesis reaction conditions. Any variation on temperature or time yielded no desired indanones, only starting materials, and often resulted in decomposition to afford a mixture of unidentifiable compounds.

4.2.3. Route 2 - Intermolecular Indanone Synthesis

The use of TFAA and TFA were again exploited when Route 2 was investigated due to its success in previous reactions. Optimisation was carried out on piperonylic acid and cyclohexene, in order to access the same indanone **136** for direct comparison (Scheme 108).



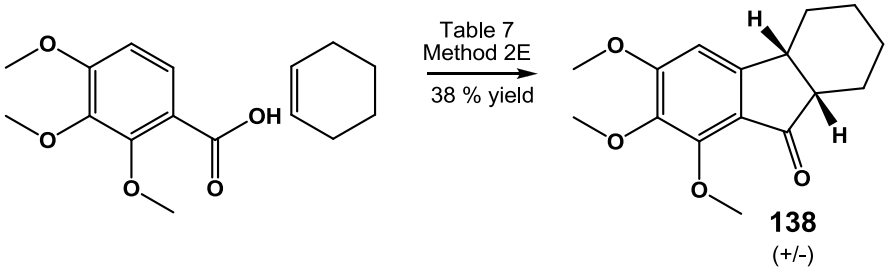
Scheme 108: Optimisation of the synthesis of indanone **136** *via* Route 2

Different equivalents of TFAA and TFA were used, the equivalents of cyclohexene were varied and the time was also altered (Table 6). Optimum conditions (Method 2C) of piperonylic acid and 1.1 eq. of cyclohexene in TFAA at 100 ^{o}C for 16 hours afforded desired indanone **136** in 51 % yield.

Method	Eq. TFAA	TFA (eq.)	Eq. cyclohexene	Time (h)	Yield %
2A	3.5	0	1.0	4	35
2B	3.5	0	1.1	6	14
2C	**3.5**	**0**	**1.1**	**16**	**51**
2D	3.5	0	2.0	17	47
2E	2.0	6.5	1.1	17	10
2F	1.0	6.5	1.1	17	18

Table 6: Optimisation of the synthesis of indanone **136** *via* Route 2 at 100 ^{o}C

2,3,4-Trimethoxybenzoic acid was then subjected to these optimised reaction conditions from Table 6 (Method 2C). However, this reaction only yielded 13 % of indanone **138** and therefore the reaction with this substrate also had to also be optimised (Table 7). Optimum conditions (Method 2E) with 1.1 eq of TFAA and addition of 0.5 mL of TFA at 100 ^{o}C for 16 hours afforded desired indanone **138** in 38 % yield (Scheme 109).

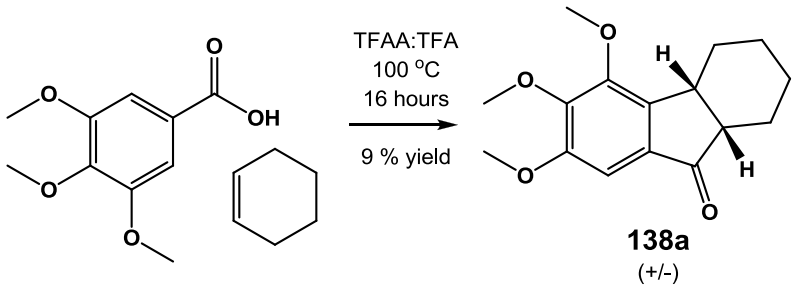


Scheme 109: Synthesis of indanone **138**

Method	Eq. TFAA	TFA (eq.)	Eq. cyclohexene	Time (h)	Yield %
2A	3.5	0	1.1	16	13
2B	3.5	0	1.1	18	16
2C	3.5	0	1.1	6	9
2D	3.5	0	1.0	24	7
2E	**1.1**	**6.5**	**2.0**	**16**	**38**

Table 7: Optimisation of the synthesis of indanone **138** *via* Route 2 at 100 ^{o}C

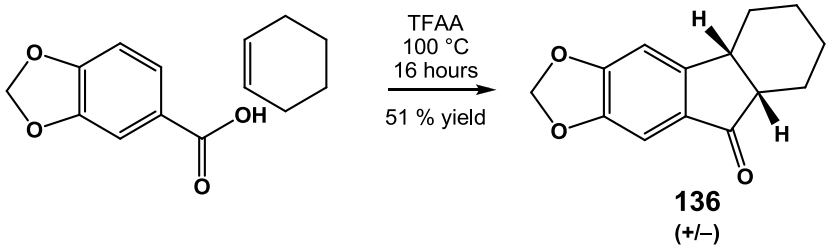
These optimised conditions (Table 7) were applied to 3,4,5-trimethoxybenzoic acid. However indanone **138a** was isolated in a reduced 9 % yield (Scheme 110).



Scheme 110: Synthesis of indanone **138a** in 9 % yield

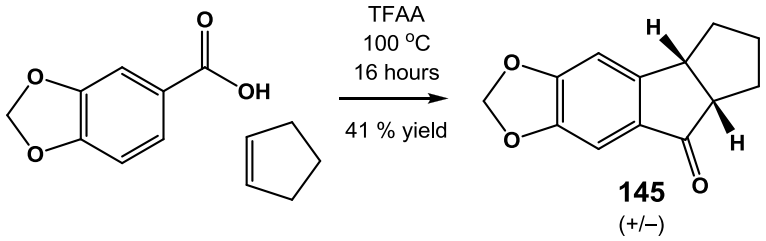
4.2.4. Route 2 - Introducing Functionalisation

The optimised conditions that used piperonylic acid and cyclohexene to afford indanone **136** in 51 % yield, shown in Scheme 111, were applied to other substituted cyclohexenes to attempt to synthesise the corresponding functionalised indanones.



Scheme 111: Optimised conditions for the synthesis of indanone **136** *via* Route 2

These optimised conditions were attempted on a variety of substrates to synthesise indanones with substituted C-rings. First cyclopentene was attempted to yield the *cis* indanone **145** in 41 % yield. (Scheme 112, see appendices **8.3.3.** for NOESY NMR).



Scheme 112: Synthesis of indanone **145**

The incorporation of other C-rings was then attempted, first with minimal functionalisation. 1,3-Cyclohexadiene, 1,4-cyclohexadiene and norbornylene were treated with piperonylic acid but the synthesis of their corresponding indanones was unsuccessful, despite varying the time and temperature of the reaction conditions (Figure 33).

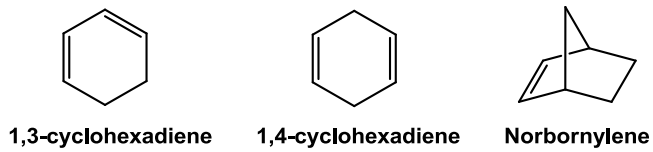
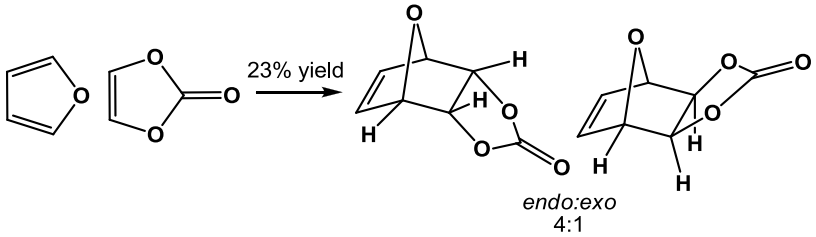


Figure 33: The unsuccessful incorporation of several functionalised C-rings

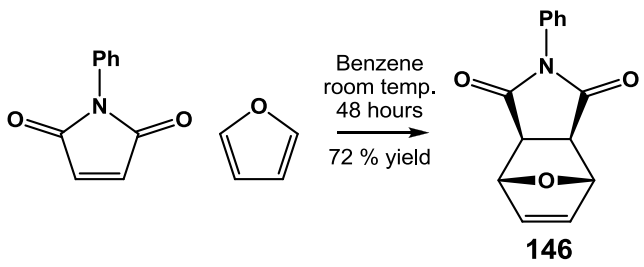
Baran *et al.* reported the successful Diels-Alder cyclisation reaction between furan and vinylene carbonate in a 4:1 ratio of *endo* to *exo* (Scheme 113).[125] This reaction was repeated, also with microwave irradiation at 200 ^{o}C and only starting material was isolated.



Scheme 113: Diels-Alder reaction between furan and vinylene carbonate[125]

Diels-Alder reactions require an electron-rich diene and electron-poor dienophile in order to proceed. Alternatively an electron-rich dienophile and electron-poor diene will react. Furan is an electron-rich diene and therefore an electron-poor dienophile is required. However, the electron density of vinylene carbonate is unclear and both arguments have previously been reported. Burnell *et al.* stated that vinylene carbonate is electron-rich[126] and Bouredeland *et al.* argued that it is electron-poor.[127]

N-Phenylmaleimide is an electron-poor dienophile and, by treating this with furan, the *exo* Diels-Alder product **146** was synthesised in 72 % yield (Scheme 114).[124] The structure of the *exo* product was confirmed using the Karplus equation.[121]



Scheme 114: Synthesis of Diels-Alder product **146** from *N*-phenylmaleimide and furan

The dihedral angle H^A-C-C-H^B for the *endo* product is 37° which will have a ^3J coupling of ~8 Hz and 81° for the *exo* product, which will have an extremely low ^3J coupling of ~0-2 Hz (Figure 34). The coupling between these two protons observed in the ^{1}H NMR for product **146** is between 0-1 Hz, which is consistent with the *exo* product.

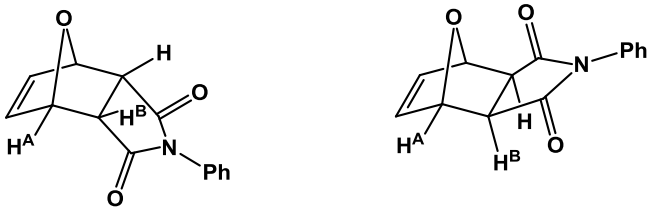


Figure 34: *Endo* and *exo* coupling

The Diels-Alder product **146** was then submitted to the conditions used previously for syntheses of the indanones but, unfortunately, the reagents decomposed during the reaction to afford a mixture of unidentifiable products.

In conclusion, the indanone synthesis is extremely limited *via* Routes 1 and 2. Any variation on either the A-ring or the C-ring resulted in diminished yields or unsuccessful reactions. To explore the second main transformation the Schmidt reaction was attempted on both isolated indanones **136** and **145** (Figure 35).

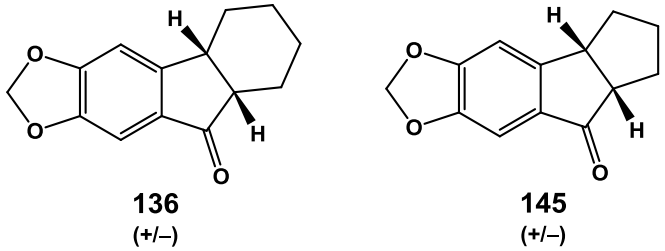
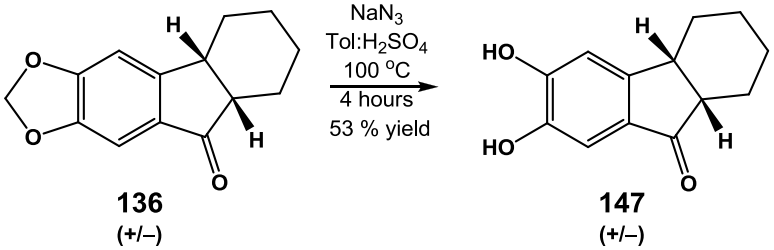


Figure 35: Indanones **136** and **145** to be used as reagents in the Schmidt reaction

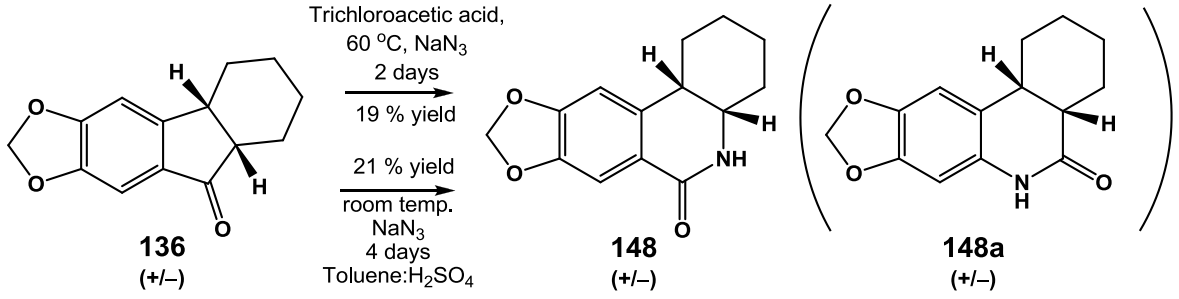
4.2.5. Schmidt Reaction and Beckmann Rearrangement

Following a procedure reported by Elderfield and Losin[128] indanone **136** and NaN_3 in H_2SO_4 and toluene at 100 ^oC afforded no desired product. However, the unexpected deprotected diol **147** was isolated in 53 % yield (Scheme 115).



Scheme 115: Synthesis of diol **147** in 53 % yield

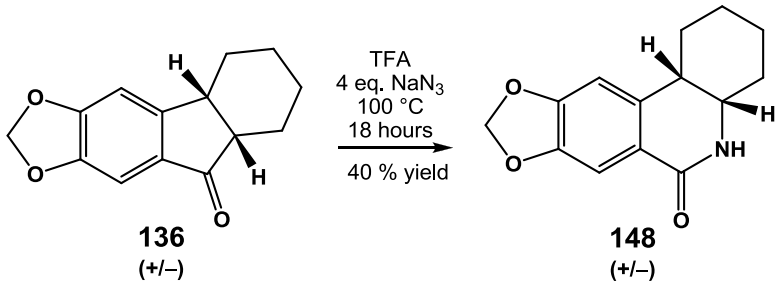
Following the conditions reported by Irie *et al.*[98], reaction of indanone **136** and NaN_3 in trichloroacetic acid for two days afforded lactam **148** in 19 % yield (Scheme 116). No lactam **148a** was observed or isolated from the reaction mixture. The *cis* configuration of the B/C ring junction and the regiochemistry of the lactam were confirmed by NOESY NMR (See appendices **8.3.4.**). Irie *et al.* also reported that even small modifications in the structure of the starting indanone can alter the success of the Schmidt reaction, which could explain the low yield of 19 %.[129]



Scheme 116: Two successful Schmidt reactions on indanone **136**

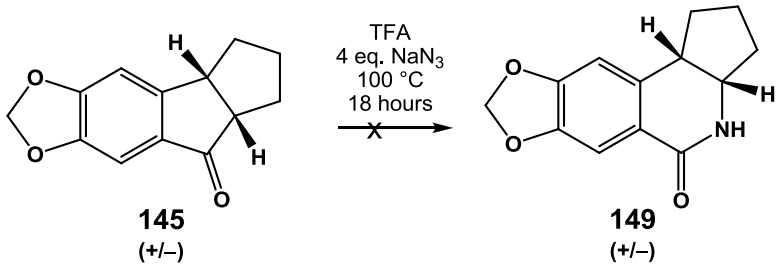
Following the procedure reported by Jesudason *et al.*[96], reaction of indanone **136** and NaN_3 in H_2SO_4 and toluene for 4 days yielded lactam **148** in 21 % yield (Scheme 116). Regioisomer **148a** was not observed.

After the success of the Schmidt reactions on A/B indanone analogues using 2 equivalents of NaN_3 in TFA at 100 ^oC for 18 hours (Scheme 69 and 76), the same conditions were investigated on A/B/C indanone **136**. However, only starting material was recovered. The alternative conditions using 4 equivalents of NaN_3 in TFA at 100 ^oC for 18 hours were then attempted, furnishing the desired regiolactam **148** in an improved 40 % yield (Scheme 117).



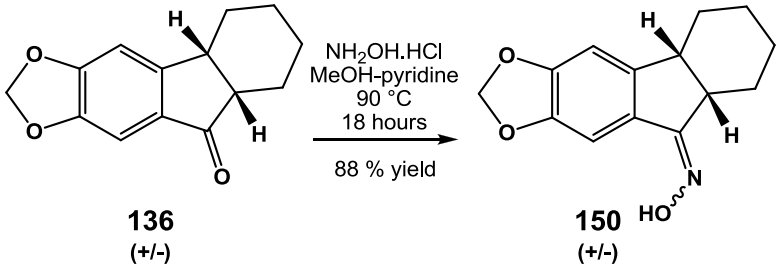
Scheme 117: Schmidt reaction to yield lactam **148** in 40 % yield

The identical procedure was attempted on indanone **145** but only starting material was recovered and no desired lactam **149** was observed (Scheme 118).



Scheme 118: Unsuccessful Schmidt reaction on indanone **145**

A Beckmann Rearrangement was also attempted on A/B/C indanone **136**. Initially the corresponding oxime **150** was synthesised using hydroxylamine in MeOH and pyridine at 90 ^oC for 18 hours in 88 % yield (Scheme 119).



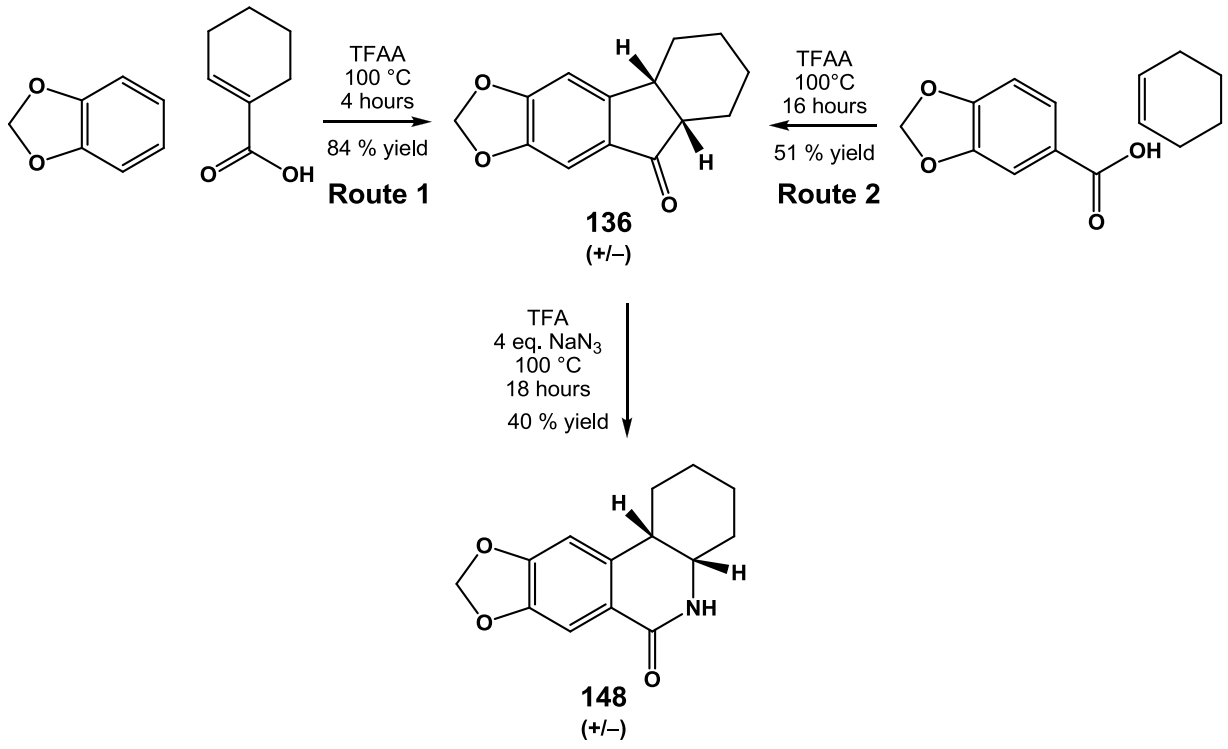
Scheme 119: Synthesis of oxime **150**

Oxime **150** in PPA at 120 ^{o}C for 2 hours gave a mixture of unidentifiable products.[130] Due to some success using TFA in the Schmidt reactions, oxime **150** was heated at 100 ^{o}C in TFA in a pressure tube for 2 hours. By TLC and NMR spectroscopy, only starting oxime or indanone were shown to remain.

4.2.6. Overview

The Schmidt reaction is low yielding, although the desired dihydroisoquinolinone core has been synthesised in just two steps. Scheme 120 outlines the optimised two step sequence to access the lactam core.

Indanone intermediate **136** can be prepared *via* two different routes. Lactam **148** can then be accessed using a Schmidt reaction in 40 % yield. Although this synthetic route is successful it is extremely limited. Any alterations or inclusion of functionality on the A-ring or C-ring cause diminished yields, or unsuccessful reactions. Therefore a new synthetic route will now be explored.

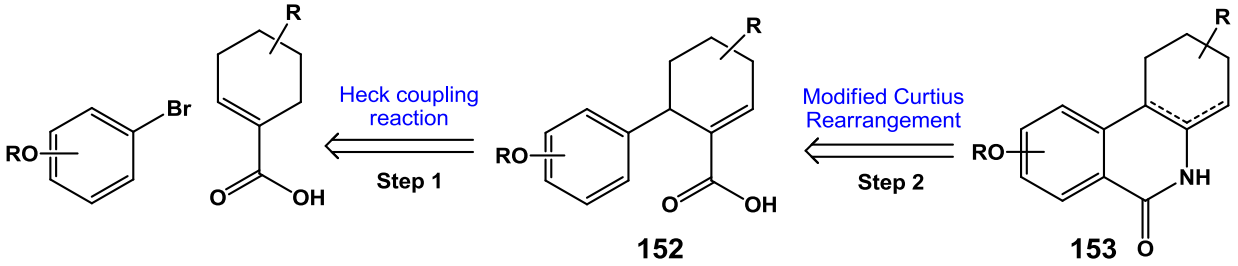


Scheme 120: Optimised two step synthesis of the dihydroisoquinolinone core

4.3. Alternative Proposed Synthetic Route

The alternative proposed synthetic route again involves two main transformations. The disconnection approach is outlined in Scheme 121.

This approach involves an initial Heck cross-coupling reaction between a substituted bromobenzene and a substituted α,β-unsubstituted carboxylic acid to afford acid intermediate **152.** The intermediate **152** can then undergo a modified Curtius rearrangement previously developed within our group[93] to potentially afford the desired A/B/C dihydroisoquinolinone ring system **153** (Scheme 121).

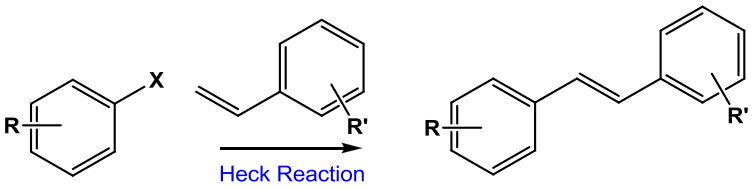


Scheme 121: Second alternative disconnection approach to access the lactam framework

4.3.1. Step 1 - Heck Cross-Coupling Reaction

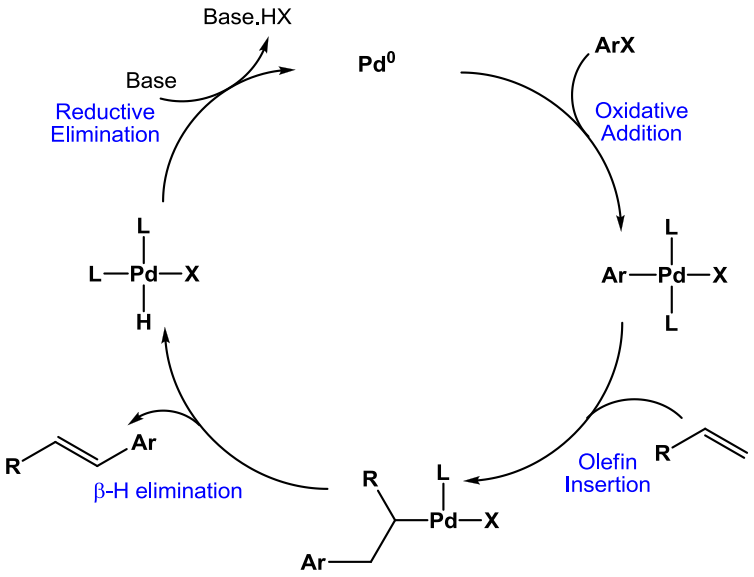
The Nobel Prize in Chemistry 2010 was awarded jointly to Richard F. Heck, Ei-ichi Negishi and Akira Suzuki "for palladium-catalyzed cross couplings in organic synthesis".[131] Palladium chemistry has become an indispensable tool for many organic chemists. The Heck-Mizoroki reaction (most commonly known as the Heck reaction) was independently discovered by both Mizoroki and Heck in the 1970s and today is a widely used palladium-catalysed cross-coupling reaction.[132]

In the Heck reaction aryl, alkenyl and benzyl halides are coupled with activated alkenes in the presence of palladium catalysts to give their corresponding *trans* substituted alkenes (Scheme 122) and is an important transformation in the synthesis of pharmaceuticals and agrochemicals.[131]



Scheme 122: Heck reaction[131]

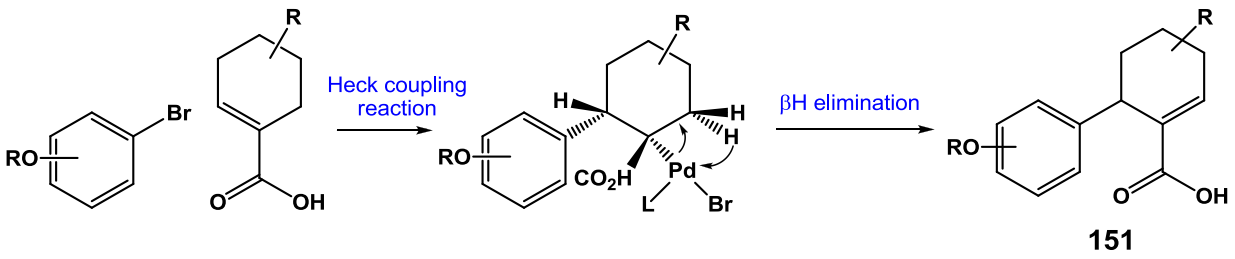
The reaction mechanism of the Heck reaction is shown in Scheme 123. The reaction starts with oxidative addition of the aryl-X compound (X can be Br, Cl, OTf, etc.) to Pd^0 to form the Pd^{II} species. Coordination and insertion of the olefin generates an alkylpalladium complex. After rotation of the C-C bond, β-H elimination occurs and the substituted alkene is released. The active Pd^0 species is reformed *via* reductive elimination by the addition of a base.



Scheme 123: Mechanism for the Heck reaction[131]

The initial Heck reaction between a substituted bromobenzene and a substituted α,β-unsaturated cyclohexene carboxylic acid will potentially yield desired acid intermediate **151** (Scheme 124). The double bond in this ring will migrate, as shown in Scheme 121, due to the constrained olefin ring system. The alkylpalladium complex formed in the mechanism is constrained in a ring and has restricted rotation; therefore for β-H elimination to occur it must

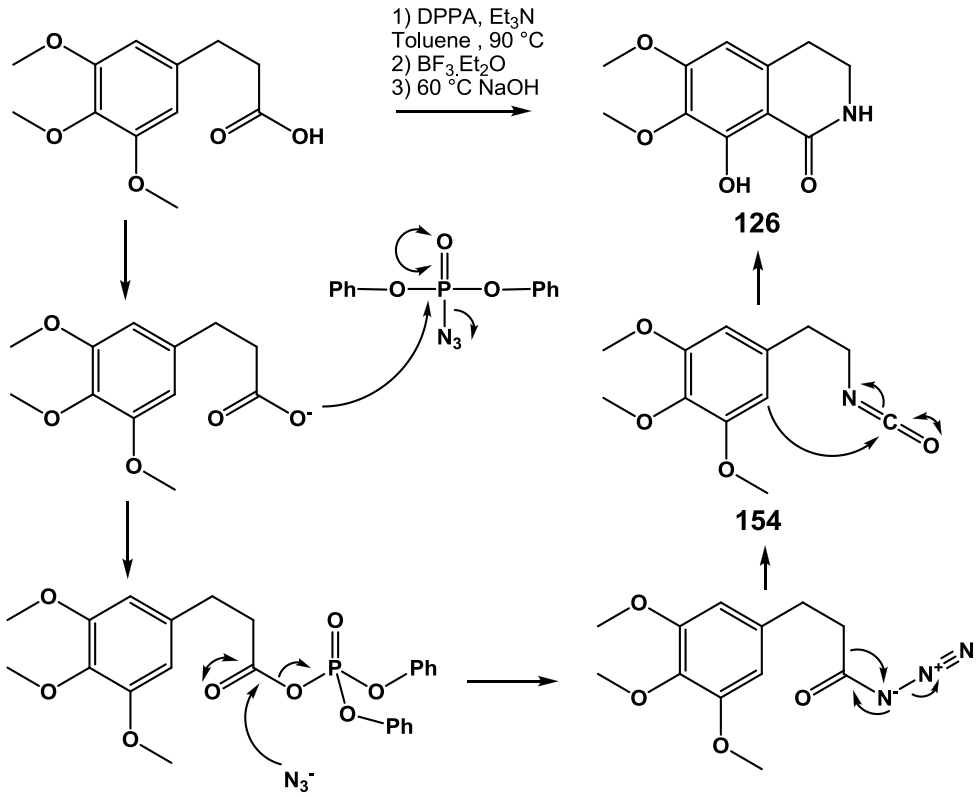
take a hydride from the neighbouring carbon, resulting in the migration of the alkene (Scheme 124).



Scheme 124: First main transformation is the Heck cross-coupling reaction

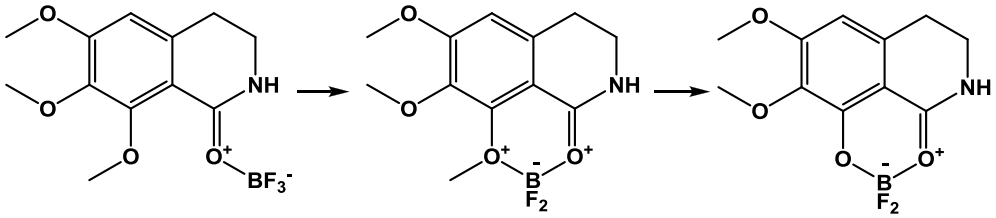
4.3.2. Step 2 - Modified Curtius Rearrangement

A successful reaction previously developed within the group is a modified Curtius rearrangement (Scheme 125).[93,110] This transformation yields the desired lactam **126** from 3-(3,4,5-trimethoxyphenyl)propanoic acid.



Scheme 125: Modified Curtius rearrangement

3-(3,4,5-Trimethoxyphenyl)propanoic acid is initially activated by DPPA which is then displaced by the azide to form the acyl azide. Rearrangement yields isocyanate **154** which is captured by the aromatic ring. Demethylation of the methoxy group occurs due to the BF_3 which initially complexes to the carbonyl oxygen (Scheme 126). This then directs the Lewis acid to form a bidentate complex with the oxygen of the 8-methoxy group. With the Lewis acid attached to to this methoxy group, a nucleophile can then attack the methyl carbon. The BF_2 complex is then hydrolysed to afford the free phenol.

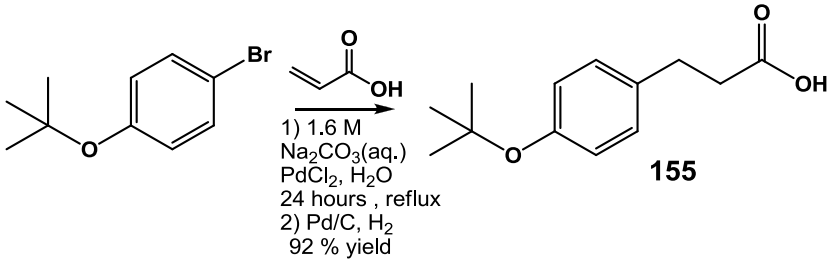


Scheme 126: Formation of BF_2 complex and subsequent demethylation of 8-methoxy group

4.4. Results and Discussion - Step 1 - Heck Reaction

4.4.1. Optimisation of Heck Reaction

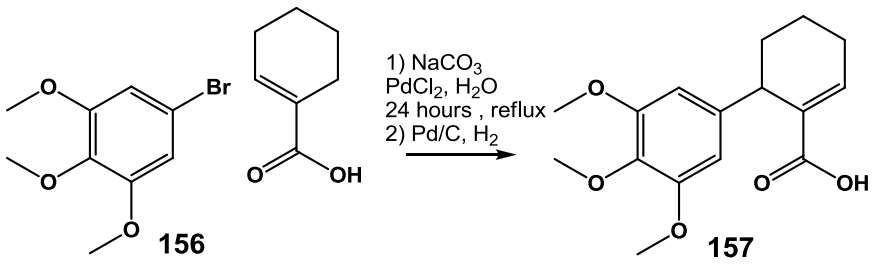
Martin *et al.*, in 2001, reported a Heck coupling reaction followed by a hydrogenation between acrylic acid and 4-*tert*-butoxy-bromobenzene to yield the desired product **155** in 92 % yield (Scheme 127).[133] The Heck coupling reaction is performed in H_2O at 100 ^oC with the addition of 1.6 M aqueous Na_2CO_3 and $PdCl_2$. The reduction of the *trans* double bond form *in situ* is then carried out in Pd on carbon under a H_2 atmosphere to yield product **155**.



Scheme 127: Reductive Heck cross-coupling reaction[133]

Unfortunately after many attempts of this reaction using 5-bromo-1,2,3-trimethoxybenzene **156** and α,β-unsaturated cyclohexene carboxylic acid as starting materials, the Heck coupling

reaction was unsuccessful (Scheme 128). A small trace of product **157** was observed in the reaction mixture; mostly, starting material was recovered.



Scheme 128: Reductive Heck cross-coupling reaction to afford a trace of compound **157**

A variety of Heck reaction conditions were then attempted on 5-bromo-1,2,3-trimethoxybenzene **156** and α,β-unsaturated cyclohexene carboxylic acid as reagents, to potentially yield desired product **157**.

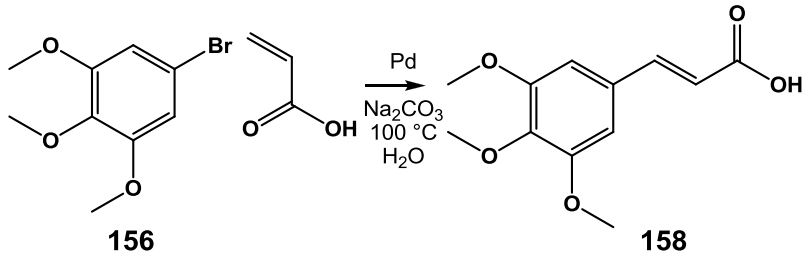
Table 8 displays a summary of the Heck reactions attempted to synthesise acid intermediate **157**. Unforunately all of these were unsuccessful. Only starting materials were seen by TLC and ^{1}H NMR spectroscopy. In each reaction, $Pd(OAc)_2$ was used as the palladium source. A possibility for the failure of these reactions could be due to catalyst inactivation.

Met-hod	**156 (eq.)**	**Acid (eq.)**	**Pd source / ligand**	**Base**	**Solvent**	**Temp (^{o}C)**	**Time (h)**	**Result**
A[134]	2	1	$Pd(OAc)_2$ (10mol%) PPh_3 (20mol%)	Na_2CO_3 (2 eq.)	DMF degassed	100	16	Starting materials
B[135]	2	1	$Pd(OAc)_2$ (10mol%)	Et_3N (2 eq.)	MeCN degassed	100	16	Starting materials
C[136]	1.5	1	$Pd(OAc)_2$ (20mol%) PPh_3 (40mol%)	Ag_2CO_3 (7 eq.)	DMF degassed	70	16	Starting materials
D[137]	2	1	$Pd(OAc)_2$ (0.3 eq.) PPh_3 (0.6 eq.)	Na_2CO_3 (5 eq.)	*t*$Bu_4NCl.H_2O$ & DMF degassed	90	16	Starting materials

E[137]	1	2	$Pd(OAc)_2$ (5mol%)	$NaHCO_3$ (4 eq.)	$tBu_4NCl.H_2O$ & DMF degassed	90	16	Starting materials
D[138]	1	2	$Pd(OAc)_2$ (5mol%) PPh_3 (20mol%)	Et_3N (1.1 eq.)	Toluene degassed	100	16	Starting materials
E	1	1	$Pd(OAc)_2$ (1mol%) PPh_3 (4mol%)	Et_3N (2.1 eq.)	Xylene degassed	100	16	Starting materials
F[139]	1	1.5	$Pd(OAc)_2$ (5mol%)	DIPEA (1.5 eq.)	tBu_4NCl DMA/H_2O degassed	120	16	Starting materials
G[140]	2	1	$(PPh_3)_2PdCl_2$ (10mol%)	Na_2CO_3 (2 eq.)	DMF Pressure tube	120	16	Starting materials

Table 8: Attempts of a Heck cross-coupling reaction to yield intermediate **157**

Due to the unsuccessful attempts of the Heck cross-coupling reactions, it was decided to return to the initial conditions using H_2O at 100 ^oC with the addition of Na_2CO_3 and $PdCl_2$ as a trace of product was seen in this reaction (Scheme 128).[133] Initially acrylic acid was explored as the starting material which was used in the reported synthesis. Only the Heck cross-coupling reaction has been explored here, without the reported reduction reaction. Scheme 129 displays the *trans* product **158** expected from this Heck reaction.



Scheme 129: Optimisation of Heck reaction to yield compound **158**

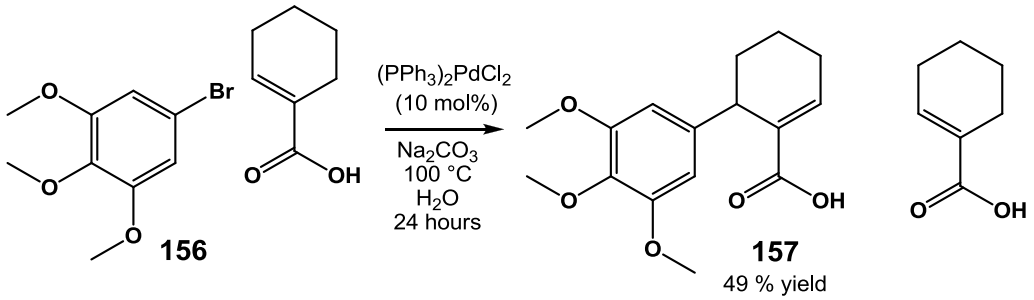
Initial reactions were carried out using three different palladium catalysts all under the same reaction conditions (Table 9).

Method	Pd Source	Na_2CO_3	Solvent	Temp (^oC)	Time (h)	Yield of 158 (%)
Method 1	**$PdCl_2$** (10mol %)	3 eq.	H_2O	100	24	13 %
Method 2	**$(PPh_3)_2PdCl_2$** (10mol %)	3 eq.	H_2O	100	24	71 %
Method 3	**$Pd(OAc)_2$** (10mol %)	3 eq.	H_2O	100	24	4 %

Table 9: Heck cross-coupling reaction using three different Pd catalysts **yields calculated by ^1H NMR spectroscopy of crude reaction mixture*

Both product **158** and acrylic acid were present in all three reaction mixtures. The yields were calculated by ^1H NMR spectroscopy of the crude reaction mixture. As shown in Table 9, Method 2 using $(PPh_3)_2PdCl_2$ as the palladium source gave the greatest yield of product **158** (71 % yield).

A reaction with an excess of bromobenzene **156,** α,β-unsaturated cyclohexene carboxylic acid and $(PPh_3)_2PdCl_2$ as the Pd source was attempted. An excess of bromobenzene **156** was used to drive the reaction to completion with any unreacted bromobenzene being removed during work-up. However, both product **157** (49 % yield) and starting material were present in the ^1H NMR spectrum.

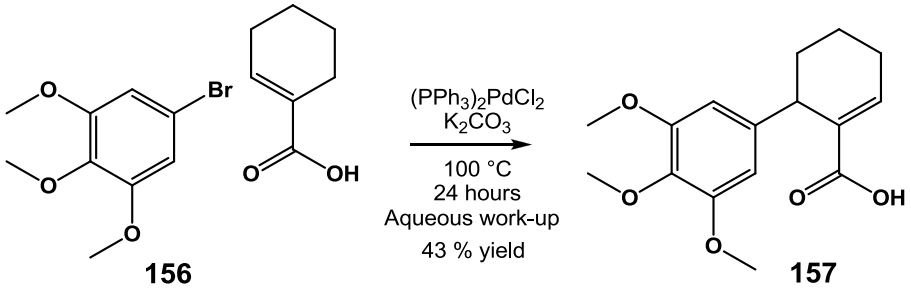


Scheme 130: Successful Heck cross-coupling reaction

Identical reaction conditions were then performed but for 48 hours rather than 24 hours, although this did not improve the yield of this reaction. The loading of the catalyst was

increased to 20mol % but again this did not improve the yield. The addition of an alternative base was then explored. The addition of Cs_2CO_3 decreased the yield of compound **157** to 20 %. However, the addition of K_2CO_3 as a base increased the yield of compound **157** by crude ^{1}H NMR to 55 %.

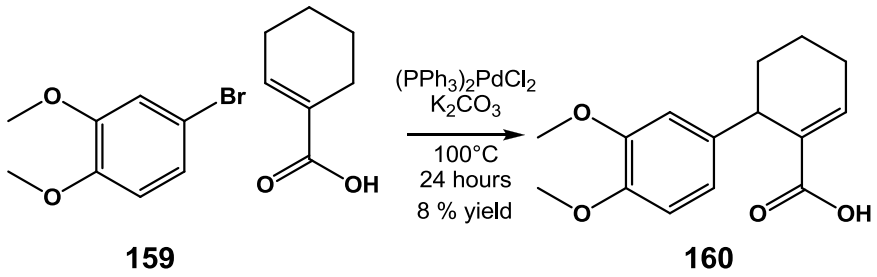
The optimised Heck reaction on trimethoxybromobenzene **156** using $(PPh_3)_2PdCl_2$ and K_2CO_3 as a base at 100 ^oC for 24 hours yielded the desired acid **157** after an aqueous work up and column chromatography in 43 % yield (Scheme 131).



Scheme 131: Heck cross-coupling reaction to afford compound **157**

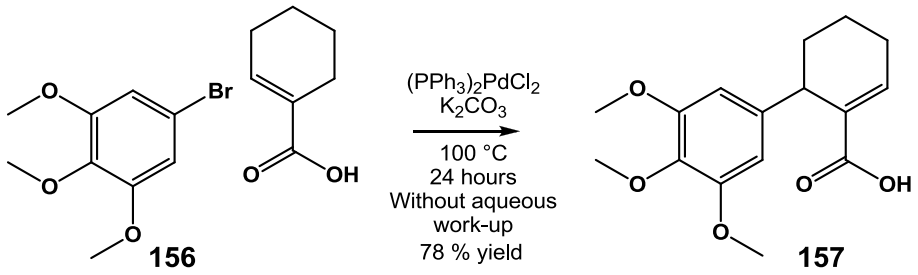
These conditions were carried out on a 2 mmol scale. The yield decreased to 33 % when the reaction was performed on a larger 4 mmol scale. Also, if the concentration was increased to 4 mL H_2O for 1 mmol of acid rather than 8 mL H_2O for 1 mmol of acid, the yield did not have a significant change at 41 %.

The optimised Heck conditions shown in Scheme 131 were then applied to 4-bromo-1,2-dimethoxybenzene **159** to yield desired acid intermediate **160** but in only 8 % yield (Scheme 132).



Scheme 132: Low-yielding Heck cross-coupling reaction

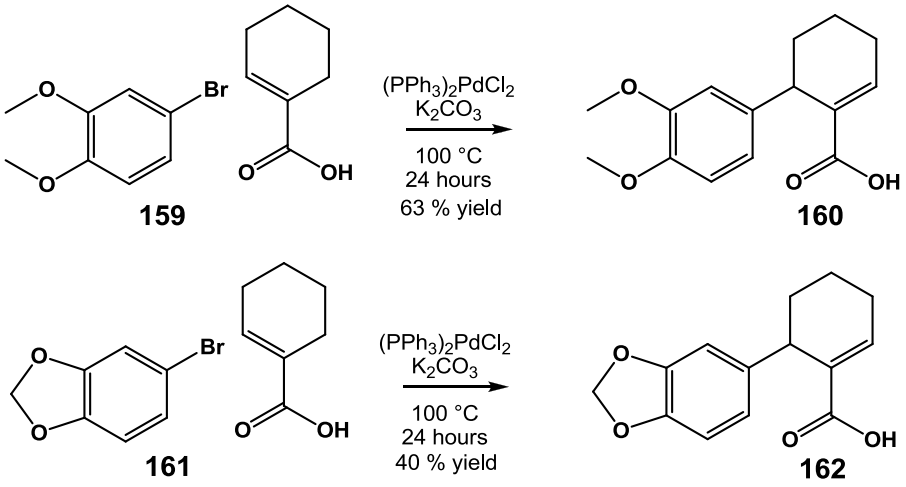
These optimised conditions using a combined basic and acidic work-up so far have been low yielding due to the difficulty of extracting out the acid. An alternative work-up was therefore attempted on the Heck reaction between bromobenzene **156** and α,β-unsaturated carboxylic acid. After 24 hours the reaction mixture was acidified, the water was removed under reduced pressure and, after column chromatography, the desired product **157** was isolated in 78 % yield (Scheme 133).



Scheme 133: Optimised Heck cross-coupling reaction to yield intermediate **157**

4.4.2. Functionalisation on A-ring

Combining the optimised Heck reaction conditions and work-up performed on 5-bromo-1,2,3-trimethoxybenzene **156** to yield intermediate **157** (Scheme 133), the conditions were then performed on 4-bromo-1,2-dimethoxybenzene **159** and 5-bromo-1,3-benzodioxole **161** (Scheme 134).The desired acid intermediates **160** and **162** were isolated in 63 % and 40 % yields respectively. The alkene has migrated in each reaction due to the mechanism of the Heck reaction.



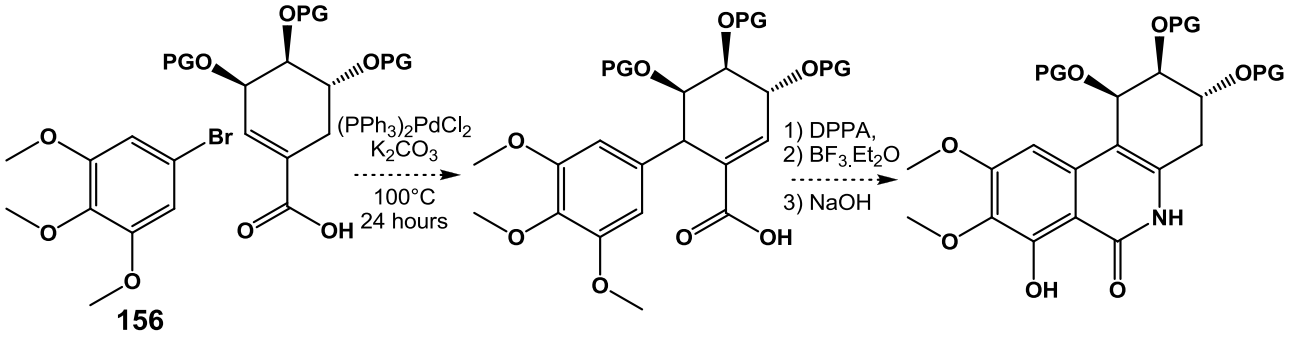
Scheme 134: Optimised Heck reaction on substituted A-rings

The modified Curtius rearrangement previously developed within the group can now be applied to these acid intermediates potentially to yield the desired lactam framework.

4.4.3. Functionalisation on C-ring

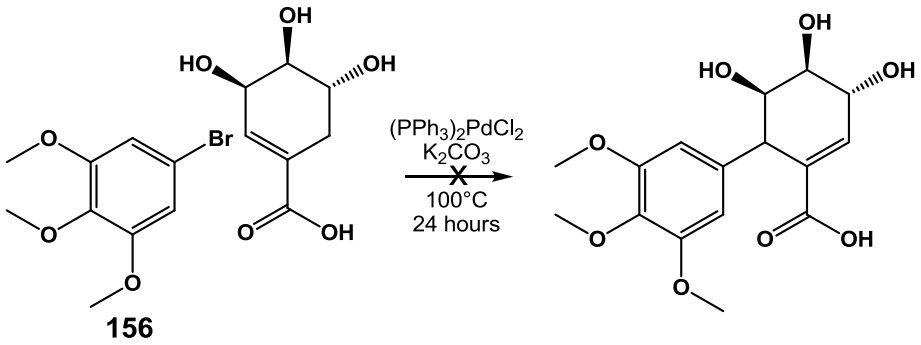
4.4.3.1 Shikimic Acid

5-Bromo-1,2,3-trimethoxybenzene **156** gave the highest yield in the Heck reaction (Scheme 133) so was therefore used as the bromobenzene when substitution on the A-ring was explored. As previously mentioned (**4.1.3.**) shikimic acid **139** is a commercially available α,β-unsaturated cyclohexene carboxylic acid which can easily be used to incorporate functionality on the C-ring (Scheme 135).



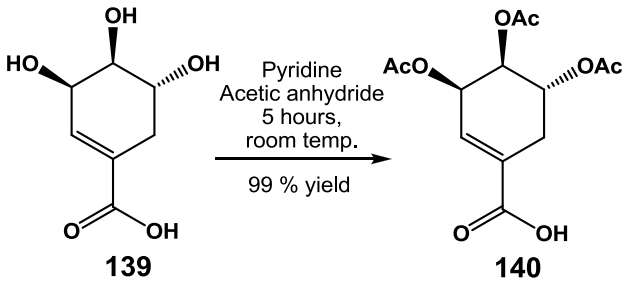
Scheme 135: The use of shikimic acid to incorporate functionality on the C-ring

The Heck reaction was initially attempted without protection of the hydroxy groups (Scheme 136). Unfortunately, no desired product was seen by NMR, only a mixture of unidentifiable compounds.



Scheme 136: Unsuccessful Heck reaction without protecting the shikimic acid

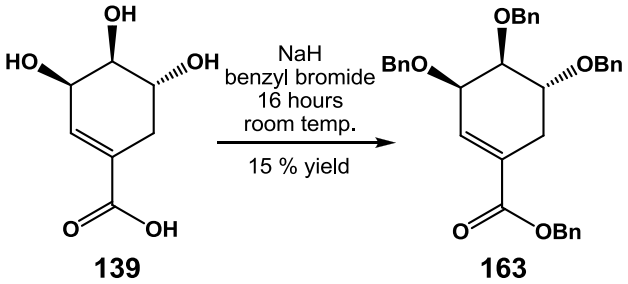
In this proposed synthetic route, the alcohol groups present on shikimic acid require protection. Protection of shikimic acid **139** with acetate groups was previously successful (Scheme 137), although these protecting groups will not be suitable for the conditions for the modified Curtius rearrangement.



Scheme 137: Protection of shikimic acid with acetate groups

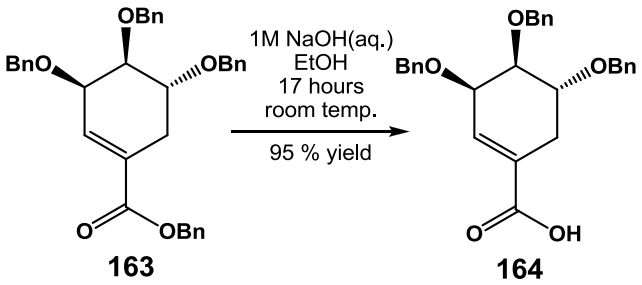
For this reason, alternative benzyl protecting groups were explored. Initial reactions to protect the hydroxy groups with a benzyl group were performed using K_2CO_3 in DMF at 80 ^oC for 24 hours[141] and another using KOH in dioxane at reflux[142] although both were unsuccessful. Mixtures of unidentifiable compounds were seen by TLC and crude ^1H NMR.

A reaction was then performed using NaH as a base.[143] The desired product **163** was isolated in a low yield of 15 % (Scheme 138).



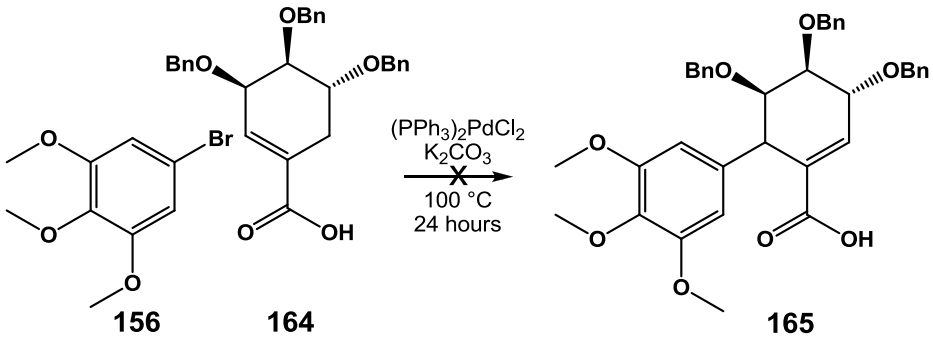
Scheme 138: Low-yielding benzyl protection of shikimic acid.[143]

This was then subjected to saponification in 1M NaOH(aq.) in EtOH at room temperature for 17 hours to yield the free acid **164** in 95 % yield (Scheme 139).[144]



Scheme 139: Hydrolysis to yield free acid **164**[144]

Protected shikimic acid was then subjected to the optimised Heck conditions to hopefully yield desired intermediate **165** (Scheme 140). Unfortunately, this was unsuccessful and ^{1}H NMR spectroscopy of the crude reaction mixture appeared to show that the benzyl protecting groups were lost under the reaction conditions.

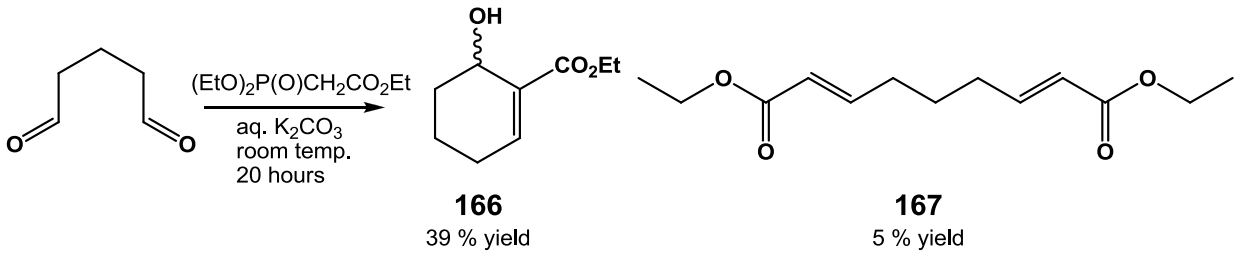


Scheme 140: Unsuccessful Heck reaction on protected shikimic acid

4.4.3.2 Mono-Oxygenated C-Ring

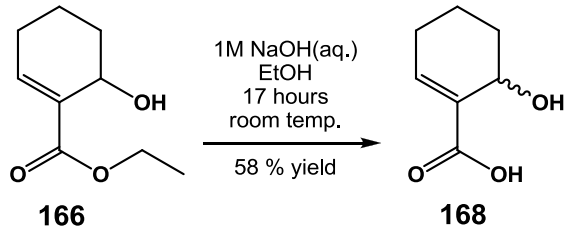
Due to the high number of hydroxy groups present on shikimic acid, a simpler mono-oxygenated C-ring was considered. This would allow optimisation of the reaction conditions in the presence of only one alcohol group, which will potentially be simpler to optimise.

The Horner-Wadsworth-Emmons reaction, followed by an aldol reaction *in situ*, was performed on glutaraldehyde using triethyl phosphonoacetate.[145] The reaction yielded the desired cyclised product **166** in 39 % yield and the di-ester product from the Horner-Wadsworth-Emmons reaction **167** was also isolated in 5 % yield (Scheme 141). Repeating this reaction on a larger scale of 20 mmol, the yield of product **166** diminished to 14 %.



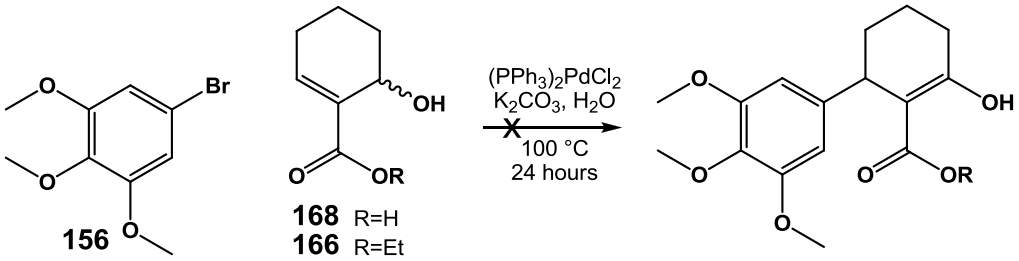
Scheme 141: Horner-Wadsworth-Emmons reaction on glutaraldehyde, followed by an aldol reaction *in situ*

Cyclised product **166** was then saponified to yield desired free acid **168** in 58 % yield (Scheme 142).[146]



Scheme 142: Saponification of ester **166**

When acid **168** was submitted to the Heck conditions, mostly starting material was recovered (Scheme 143). This reaction may have been unsuccessful due to the alcohol possibly forming the ketone by keto-enol tautomerisation.



Scheme 143: Unsuccessful Heck cross-coupling reaction on 5-Bromo-1,2,3-trimethoxybenzene **156** and acid or ester **168/166**

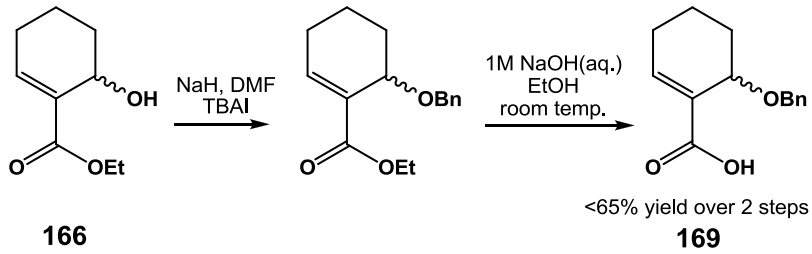
Ester **166** was also subjected to the Heck reaction; however, this again was unsuccessful. Mixtures of starting material and unidentifiable compounds were isolated (Scheme 143).

Attempts to protect the mono-oxygenated ring **166** were then made, although this proved very difficult to achieve. Initial reaction conditions include 2 equivalents of benzyl bromide

with the addition of NaOH in a mixture of H_2O and EtOH at 80 ^oC for 22 hours.[147] Unfortunately, the benzyl bromide and EtOH reacted to form ethoxybenzene.The reaction of ester **163** with NaH and benzyl bromide in THF at room temperature for 22 hours was then attempted and only starting material was isolated from this reaction.

Alternative reaction conditions using Ag_2O and benzyl bromide in DMF at room temperature for 16 hours was explored; however, only starting material and a mixture of unidentifiable compounds were isolated.[148] Experiments using ester **163** Ag_2O and benzyl bromide at room temperature for 16 hours in Et_2O or $CHCl_3$ were performed.[149] Both reactions gave starting material and no desired product.

An alternative reaction using NaH in DMF with the addition of TBAI was attempted[150] followed by saponification. Acid **169** was synthesised in less than 65 % over two steps. The saponification was a very low yielding raction so only small amounts of the desired benzyl protected free acid **169** was present by ^1H NMR (Scheme 144).



166 **169**

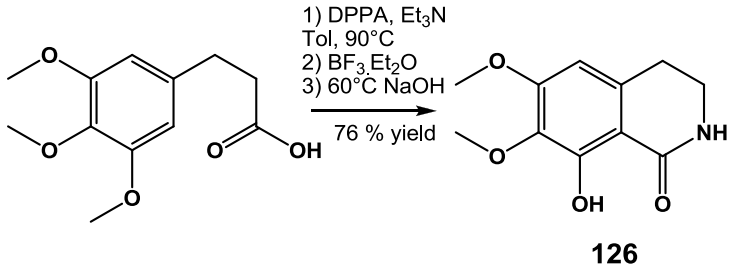
Scheme 144: Protection of ester **166** followed by saponification to yield acid **169**

Alternative protecting groups must be explored. Once the mono-oxygenated C-ring and shikimic acid have both been protected with the appropriate protecting group this proposed strategy to access the desired lactam core should be achievable.

4.5. Results and Discussion - Step 2 - Modified Curtius Rearrangement

The second main transformation in this alternative proposed synthetic route involves a modified Curtius rearrangement previously developed within our group. An initial reaction

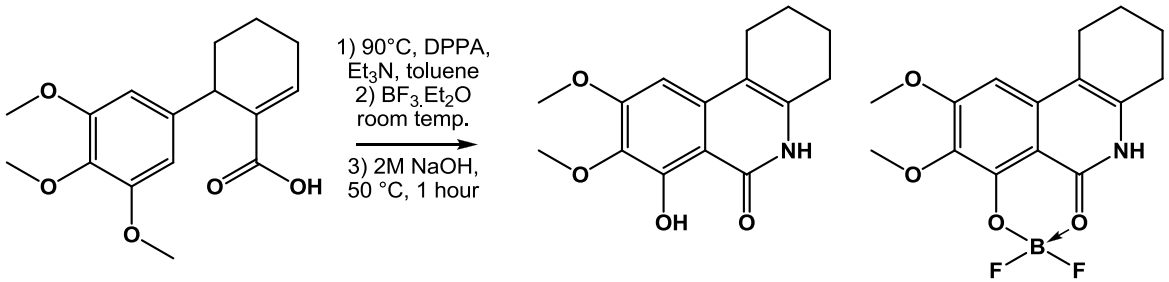
using 3-(3,4,5-trimethoxyphenyl)propanoic acid afforded lactam **126** in 76 % yield (Scheme 145).[93] The mechanism of this reaction is summarised in Scheme 125 and Scheme 126.



126

Scheme 145: Successful modified Curtius rearrangement to yield lactam **126** in 76 % yield

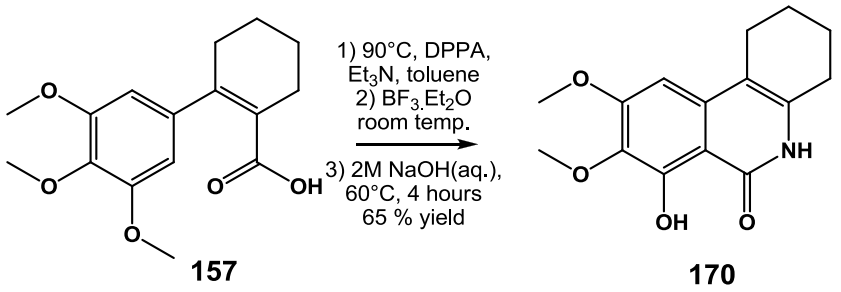
These reaction conditions were then attempted on Heck product **157**. The desired lactam **170** was isolated in 21 % yield and the BF_2 complex **171** was isolated in 39 % yield, with the double bond present in the C-ring moving back into conjugation (Scheme 146).



157 **170** 21 % yield **171** 39 % yield

Scheme 146: Modified Curtius rearrangement to yield lactam **170** in 21 % yield

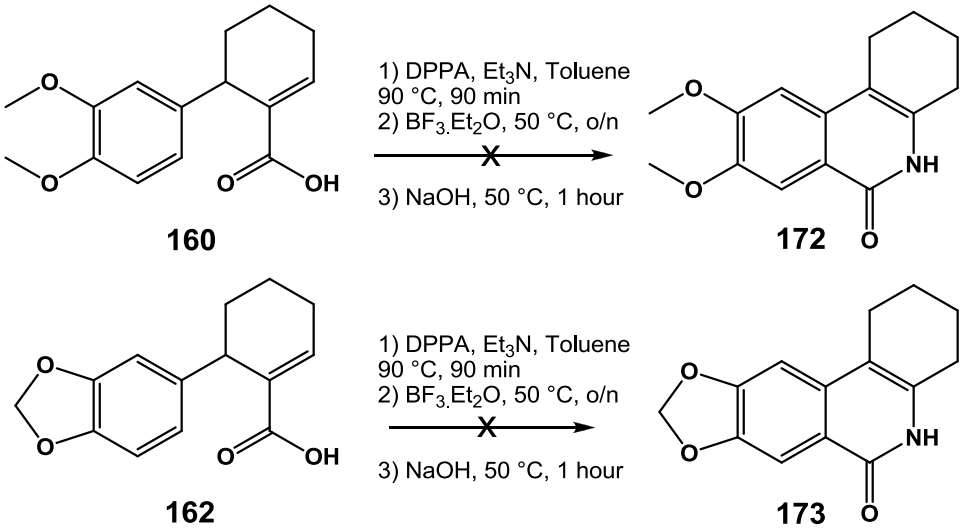
This was a promising result as the BF_2 complex can be hydrolysed further to then isolated desired lactam **170** in a greater yield. The reaction was repeated and the third hydrolysis step was heated to 60 ^{o}C for 4 hours to yield the desired lactam **170** in an improved 65 % yield (Scheme 147).



Scheme 147: Modified Curtius rearrangement to yield lactam **170** in 65 % yield

The effect of these conditions was then investigated on both Heck products **160** and **162**. Unfortunately, these reactions did not yield the desired lactams **172** and **173** (Scheme 148).

As previously mentioned, the presence of the free phenol on the A-ring increases the activity of these compounds. The failure of the modified Curtius rearrangement on intermediates **160** and **162**, which do not contain the free phenol, is only a minor setback to the overall synthetic route as these are not expected to be as active as lactam **170**. Once the mono-oxygenated C-ring is protected, the Heck reaction can be performed using 5-bromo-1,2,3-trimethoxybenzene **156** which is the most reactive A-ring reagent in the modified Curtius rearrangement.

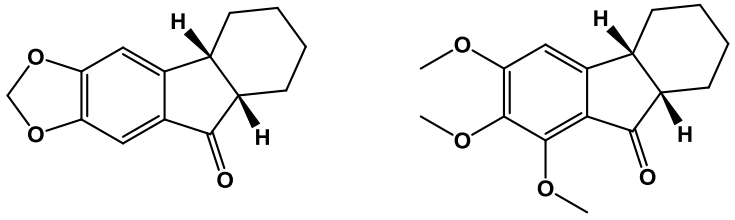


Scheme 148: Unsuccessful modified Curtius rearrangements in compounds **160** and **162**

4.6. Biological Activity

The A/B/C indanones and lactams have have also been tested in an MTS cell proliferation assay using colon cancer cell line HT29.

A/B/C indanones **136** and **138** were both tested for their biological activity against the HT29 cell line. Indanone **138** had a greater activity with an IC_{50} value of 221 μM, although this is not highly active (Figure 36).

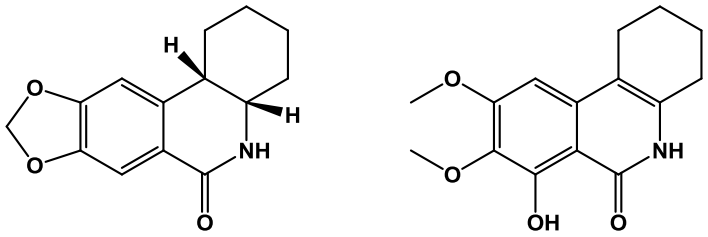


136
(+/-)
HT29 IC_{50} = >500 μM

138
(+/-)
HT29 IC_{50} = 221 μM

Figure 36: Low activity of A/B/C indanones against HT29 cell line

The two synthesised A/B/C lactams **148** and **170** were also tested against the HT29 colon cancer cell line. The corresponding A/B lactam **101** of A/B/C lactam **148** has an IC_{50} value of >500 μM in the HT29 cell line (Figure 23). Lactam **148** contains the C-ring but also has an IC_{50} value of >500 μM. Both lactams **101** and **148** lack the free phenol so the low biological activities are as expected (Figure 37).



148
(+/-)
HT29 IC_{50} = >500 μM

170
HT29 IC_{50} = 9 μM

Figure 37: IC_{50} values of A/B/C lactams **148** and **170** in HT29 colon cancer cell line

Lactam **170** contains both the C-ring and the free phenol. It also contains the double bond which has previously been shown to increase the activity. Its corresponding A/B lactam **127**

has an IC_{50} value of 71 μM in HT29 cells (Figure 23). Lactam **170** has an IC_{50} value of 9 μM in the HT29 cell line. This is the most biologically active analogue synthesised to date.

4.7. Conclusions

The aim of the work described within this chapter was to synthesise a range of A/B/C indanones and subsequently transform them into their corresponding A/B/C lactams. A range of reaction conditions were used to attempt this synthesis and largely proved unsuccessful. The synthesis of A/B/C indanones was achieved using similar reaction conditions to those for A/B indanones described in Chapter 3; however the synthesis was sensitive to temperature and reaction time. Any alterations of functionality on the A-ring or C-ring diminished the yield or rendered the reactions unsuccessful. Therefore, each A/B/C indanone required tailored reaction conditions, in terms of reaction time and temperature, to produce any product in reasonable yield. A single A/B/C lactam was reliably synthesised *via* a Schmidt reaction.

The previous conditions were limited in their ability to synthesise a library of A/B/C lactams and so a new synthetic route was explored. The new proposed synthetic route was two steps; the first step was a Heck reaction using a Pd catalyst and commercially available starting materials and the second step was a modified Curtius rearrangement. This route was used to synthesis an A/B/C lactam, **170**, containing phenol functionality on the A-ring and an unfunctionalised C-ring. This is a novel and interesting synthetic route to dihydroisoquinolinones and could be further explored to incorporate functionality on the C-ring.

Several of the compounds described in this chapter were tested for biological activity against the HT29 colon cancer cell line. Lactam **170**, a dihydroisoquinolinone, showed promising results with an IC_{50} value of 9 μM. Lactam **170** lacks functionality on the C-ring; if functionality is incorporated then biological activity should further improve.

5. Indanocine

5.1. Introduction

As previously mentioned in section **3.1.1.** many natural products which contain the indanone framework have been shown to possess biological activity.[5] Indanocine **76** is an example of a biologically active compound which contains the indanone moiety within its structure (Figure 38).[87]

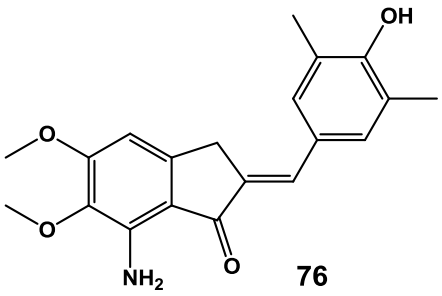


Figure 38: Indanocine **76**

Indanocine has an IC_{50} value of 0.001 μM against the Jurkat cell line.[87] It has been shown to bind to microtubules at the colchicine site that blocks tubulin polymerisation[152] and it selectively induces apoptosis in multi-drug resistant cancer cells.[88]

A screen of indanocine **76** was performed by the NCI in 49 cancer cell lines. The average GI_{50} value was less than 20 nM and the antiproliferative concentrations were lower in the multidrug-resistant cells than in the corresponding parent cells (three examples are shown in Table 10).

Cell Line	**Wild type (GI_{50})**	**Multidrug resistant (GI_{50})**
MCF-7 (human breast)	20 ± 5 nM	4 ± 1 nM
MES-SA (human uterine)	85 ± 6 nM	12 ± 3 nM
HL-60 (human leukaemia)	40 ± 3 nM	2 ± 0.2 nM

Table 10: Growth-inhibitory concentrations of indanocine **76**[88]

Indanocine had poor activity in several animal models possibly due to its poor aqueous solubility.[151]

5.2. Aim

As previously mentioned in section **1.2.3.4.** Rinner *et al.* explored the replacement of the core with an indole moiety to synthesise analogues with spatial similarities which would hopefully mimic the anticancer activity of pancratistatin (Figure 39).[52] Pancratistatin **16** displayed promising anticancer activity in the cell lines with IC_{50} values ranging from 0.062 µM - 0.138 µM. In comparison, compound **29** unfortunately displayed only slight activity in P388 lymphocytic leukaemia cell and no activity in the other three cell lines.[52]

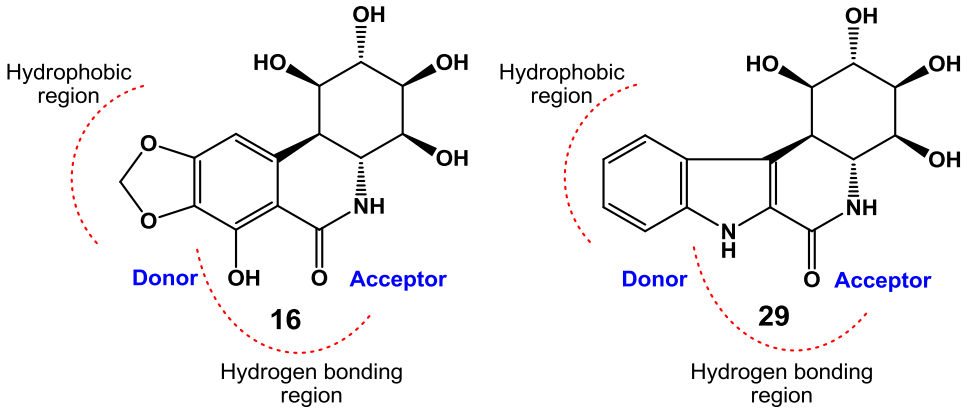


Figure 39: Replacement of the lactam core with an indole moiety[52]

The aim of this project is to apply these same principles to indanocine **76**. Using the same rationale as Rinner, a range of potential novel analogues are proposed (Figure 40). It is hoped that by substituting a primary amine for a secondary amine will create a better bioisostere than substituting an alcohol with a secondary amine as Rinner did.

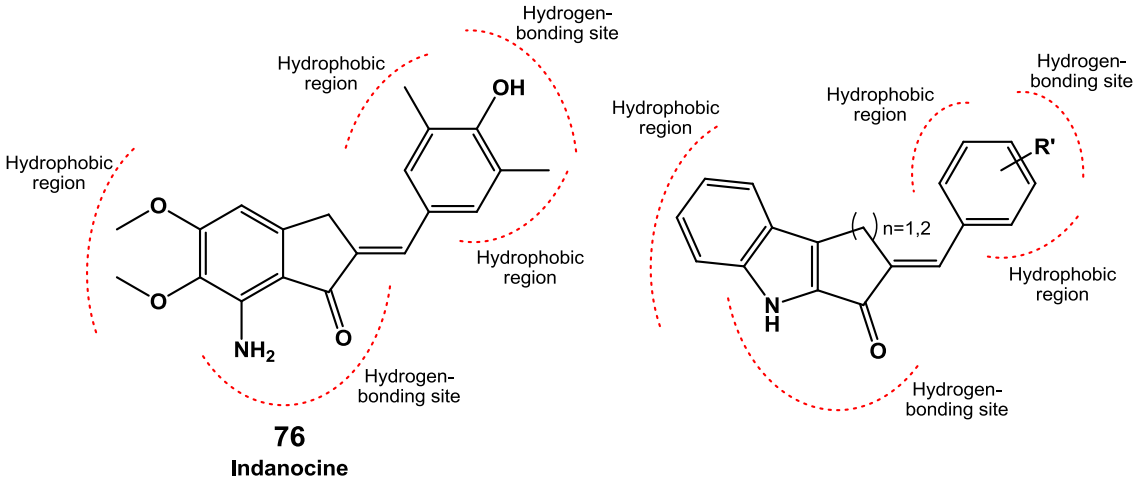
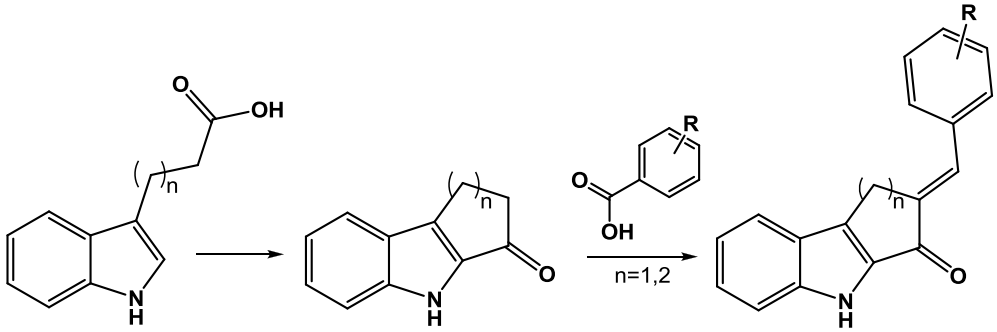


Figure 40: Indole analogue of indanocine **76**

5.3. Synthetic Route and Proposed Analogues

The proposed synthetic route involves two synthetic steps (Scheme 149). The first is an intramolecular Friedel-Crafts acylation reaction to afford the desired indanone intermediate, as previously synthesised and described in Chapter 3. The second transformation involves a Claisen-Schmidt condensation reaction using a variety of aldehydes to afford a range of desired analogues. Theoretically the *E* and *Z* geometric isomers can equally be formed in this aldol condensation reaction however the *Z* configuration is highly unfavourable due to the stong steric interactions between the aryl and the carbonyl group.[152]



Scheme 149: Proposed synthetic route towards the synthesis of indanocine analogues

For potential optimum activity, positions 3' and 5' should be substituted with hydrophobic groups. Aldehydes which contain the hydroxy group at position 4' and hydrophobic regions at positions 3' and 5' were explored, including 4-hydroxy-3,5-dimethylbenzaldehyde and 4-hydroxy-3,5-dimethoxybenzaldehyde (Figure 41).

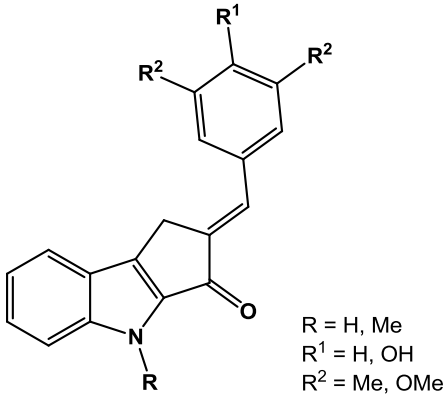


Figure 41: Proposed 5-membered ring analogues of indanocine

It was also decided to investigate the effect of the hydroxy group at position 4'. The hydroxyl group was removed with the use of aldehydes 3,5-dimethylbenzaldehyde and 3,5-dimethoxybenzaldehyde. It was also decided to examine the effect of the protection of the indole nitrogen with a methyl group compared to the free nitrogen.

Hallgas *et al.* investigated the antiproliferative activity profiling of 2-benzylidenecycloalkanones (Figure 42).[152] They discovered that the preferred most active core structure is the cyclohexanone.

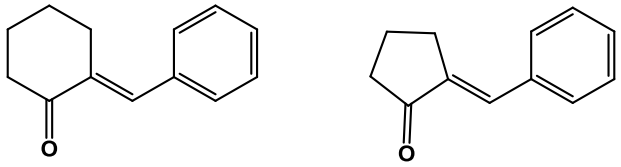


Figure 42: 2-Benzylidenecycloalkanones

Therefore 6-membered analogues were also synthesised to determine if these are more active (Figure 43).

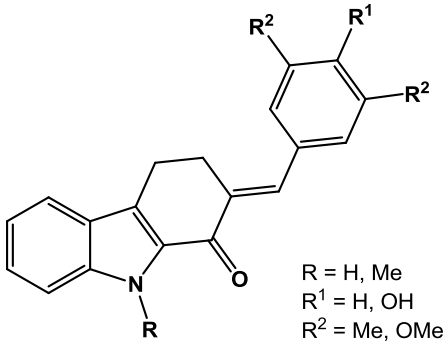
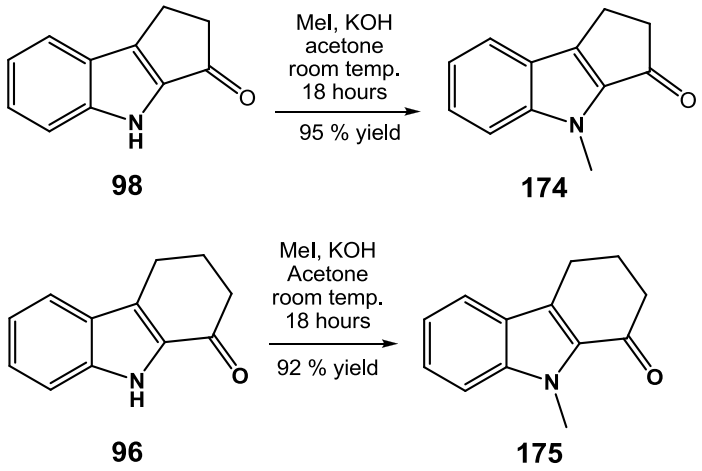


Figure 43: Proposed 6-membered ring analogues of indanocine

5.4. Synthesis of Analogues

Using cyclic ketones previously synthesised as part of the narciclasine and pancratistatin project described in Chapter 3, compounds **96** and **98** were used to synthesise novel indanocine analogues. Methylation reactions were performed on indanones **96** and **98** following the procedure reported by Judd *et al.*[93] to afford desired methylated intermediates **174** and **175** in a 95 % and 92 % yield respectively (Scheme 150).



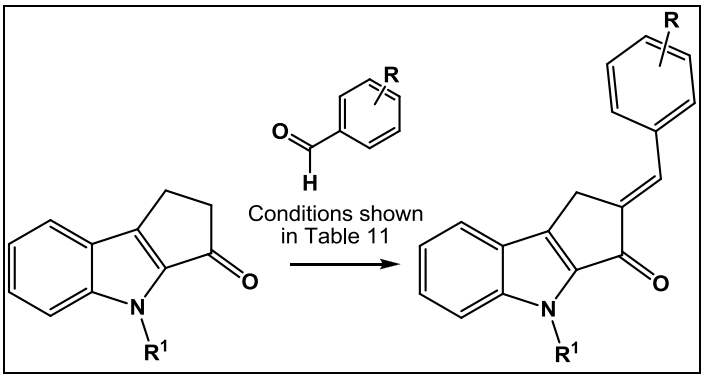
Scheme 150: Methylation of indoles **98** and **96**[93]

Reactions were then performed using a range of aldehydes shown in Table 11 with compound **98** and methylated compound **174** to afford a wide range of 5-membered ring analogues.

A large range of conditions were studied to synthesise these analogues, including NaOMe in MeOH at room temperature,[153] NaOEt in THF at room temperature,[154] Na_2CO_3 in H_2O at 70 ^oC,[155] $LiOH.H_2O$ in EtOH at room temperature,[156] 3 % ethanolic NaOH at room temperature[157] and in solvent free conditions by grinding in the presence of NaOH.[158]

Following conditions using 4 % ethanolic KOH at room temperature for 16 hours, desired analogues were synthesised in good yields (shown in Table 11).[159]

The alternative reaction using 2-methoxyethanol as the solvent with >99 % aqueous KOH solution at 120 ^oC for 20 hours afforded the desired analogues in good yields. The use of 2-methoxyethanol as a solvent was previously demonstrated in an aldol condensation reaction within the group, which is the reason reactions using this unusual solvent were attempted.



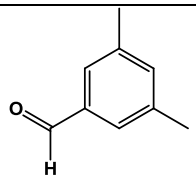
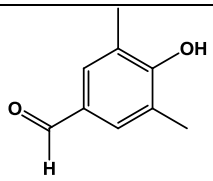
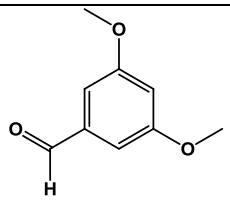
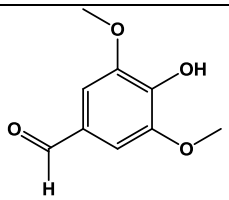
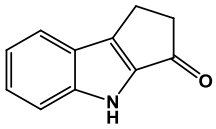
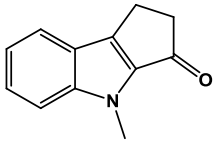
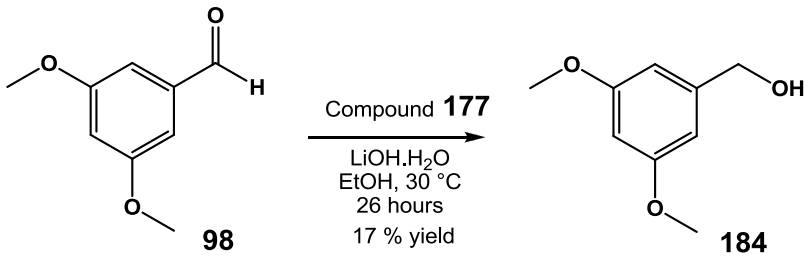
				
98 	**176** 4 % (w/v) ethanolic KOH, room temp. 16 hours **71 %**	**177** > 99 % aq. KOH solution in 2-methoxyethanol, 120 ^{o}C, 20 hours **61 %**	**178** 4 % (w/v) ethanolic KOH, room temp. 16 hours **72 %**	**179** > 99 % aq. KOH solution in 2-methoxyethanol, 120 ^{o}C, 20 hours **54 %**
174 	**180** 4 % (w/v) ethanolic KOH, room temp. 16 hours **45 %**	**181** > 99 % aq. KOH solution in 2-methoxyethanol, 120 ^{o}C, 20 hours **3 %**	**182** 4 % (w/v) ethanolic KOH, room temp. 17 hours **12 %**	**183** > 99 % aq. KOH solution in 2-methoxyethanol, 120 ^{o}C, 20 hours **49 %**

Table 11: Conditions for condensation reactions

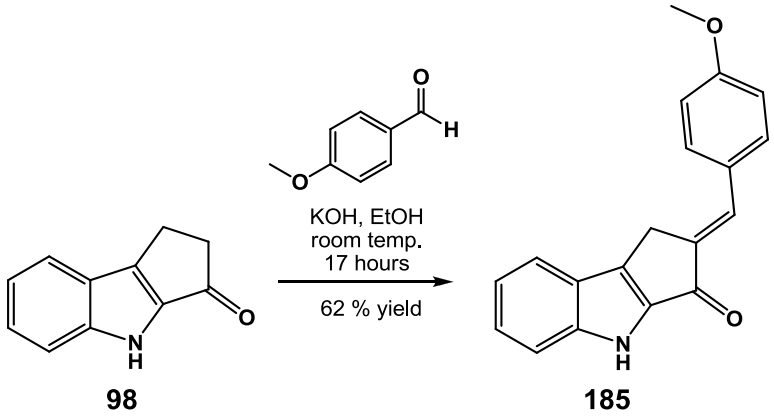
Compound **182** was synthesised in a low 12 % yield using 4 % ethanolic KOH at room temperature for 17 hours,[159] which were the most successful reaction conditions for this analogue. Analogue **181** was synthesised in the lowest yield of 3 % using 2-methoxyethanol and saturated KOH at 120 ^{o}C for 20 hours, which were the only reaction conditions to provide this analogue.

The formation of analogue **177** was attempted using $LiOH.H_2O$ in EtOH at 30 ^oC for 26 hours[156] but no desired product was observed. Only the reduced alcohol **184** was isolated in 17 % yield, which is caused by a Cannizzaro reaction (Scheme 151).



Scheme 151: Reduction of aldehyde by cannizzaro reaction to yield alcohol **184**

Compound **185** was also synthesised in 62 % yield using 4 % ethanolic KOH at room temperature for 17 hours. This analogue was also explored due to the availability of the aldehyde and the methoxy group at the 4' position rather than a hydroxy group (Scheme 152).

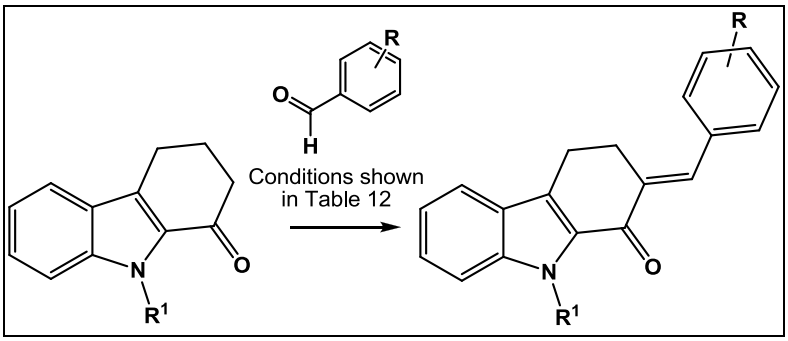


Scheme 152: Synthesis of analogue **185**

Reactions were then performed using a range of aldehydes shown in Table 12 with ketone **96** and methylated compound **175** to afford a wide range of 6-membered ring analogues.

The use of 6-membered cyclic ketones **96** and **175** gave lower yields when aldol condensation reactions were performed with the same range of aldehydes. Previous conditions using KOH were unsuccessful on these analogues; therefore, other conditions were investigated (Table 12). Following a procedure reported by Bhagat *et al.*,[156] $LiOH.H_2O$

in EtOH was used to synthesise many analogues. $BF_3.OEt_2$ was also utilised to yield desired analogues under acidic conditions.[160]



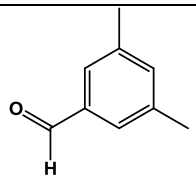
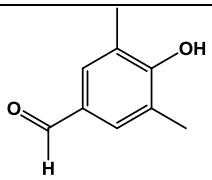
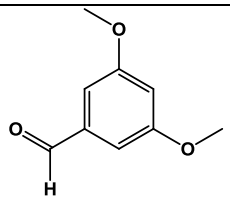
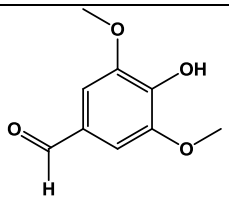
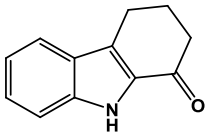
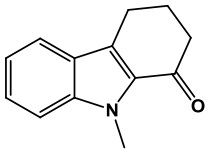
				
96 	**186** $LiOH.H_2O$ in EtOH, 30 °C 21 hours **32 %**	**187** $BF_3.OEt_2$ dioxane , 60 °C 6 hours **21 %**	**188** $LiOH.H_2O$ in EtOH, 30 °C 6 hours **52 %**	**189** $BF_3.OEt_2$ dioxane , 70 °C 21 hours **33 %**
175 	**190** $LiOH.H_2O$ in EtOH, 30 °C 20 hours **55 %**	**191** $BF_3.OEt_2$ dioxane , 60 °C 14 hours **11 %**	**192** 4 % (w/v) ethanolic KOH, room temp. 16 hours **28 %**	**193** Not isolated

Table 12: Conditions for condensation reactions

5.5. Biological Activity

The library of analogues were then tested in an MTS cell proliferation assay using colon cancer cell line HT29 and breast cancer cell line MDA231.

The results are displayed in Table 13. The most active analogues in the HT29 colon cancer cell line are the 6-membered cyclic ketones **186**, **187** and **191,** where all three analogues have an IC_{50} value of 6 μM. Analogues **186** and **187** contain the free indole nitrogen and analogues

187 and **191** contain the free phenol. However, all the 5-membered cyclic ketones tested had low activity in the HT29 colon cancer cell line.

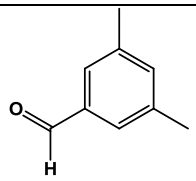
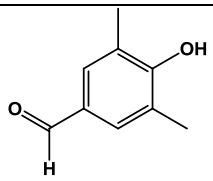
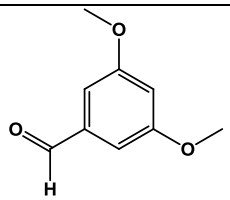
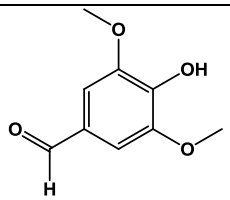
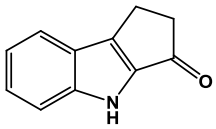
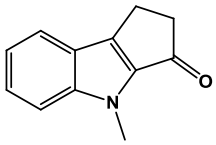
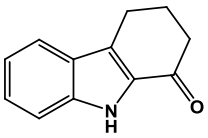
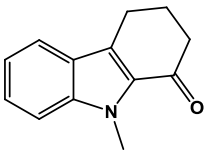
				
98 	**176** HT29 IC_{50} = >500 μM	**177** Not tested	**178** HT29 IC_{50} = >500 μM	**179** HT29 IC_{50} = >500 μM
174 	**180** HT29 IC_{50} = >500 μM MDA231 IC_{50} = >500 μM	**181** Not tested	**182** Not tested	**183** HT29 IC_{50} = >500 μM
96 	**186** HT29 IC_{50} = 6 μM MDA231 IC_{50} = 5 μM	**187** HT29 IC_{50} = 6 μM MDA231 IC_{50} = 33 μM (+/- 3 SEM)	**188** MDA231 IC_{50} = >500 μM	**189** Not tested
175 	**190** HT29 IC_{50} = 20 μM MDA231 IC_{50} = >500 μM	**191** HT29 IC_{50} = 6 μM	**192** HT29 IC_{50} = >500 μM MDA231 IC_{50} = >500 μM	**193** Not isolated

Table 13: IC_{50} values of indanocine analogues in HT29 and MDA231 cancer cell lines

The most active analogue in the breast cancer cell line MDA231 is compound **186** with an IC_{50} value of 5 μM. The second most active analogue **187** has an IC_{50} value of 33 μM. It is interesting that the two most active compounds tested are compounds **186** and **187,** which are

structurally similar. The only difference between the two compounds is the presence of the free phenol in **187**. Other analogues which were tested all possessed low activity.

5.6. NCI 60-Cell Line Screen

5.6.1. One-Dose Data

The National Cancer Institute has a service which tests the activity of compounds for potential anticancer activity by testing them in 60 different human tumour cell lines. The NCI screens up to 3000 compounds per year and the testing is performed in two parts. Firstly, a single concentration of 15μg mL^{-1} is tested in all 60 cell lines and if the results from this initial screen meet the selection criteria, then a second five-dose test is performed in all 60 cell lines, which measures the GI_{50} value, the TGI (total growth inhibition) and the LC_{50}.

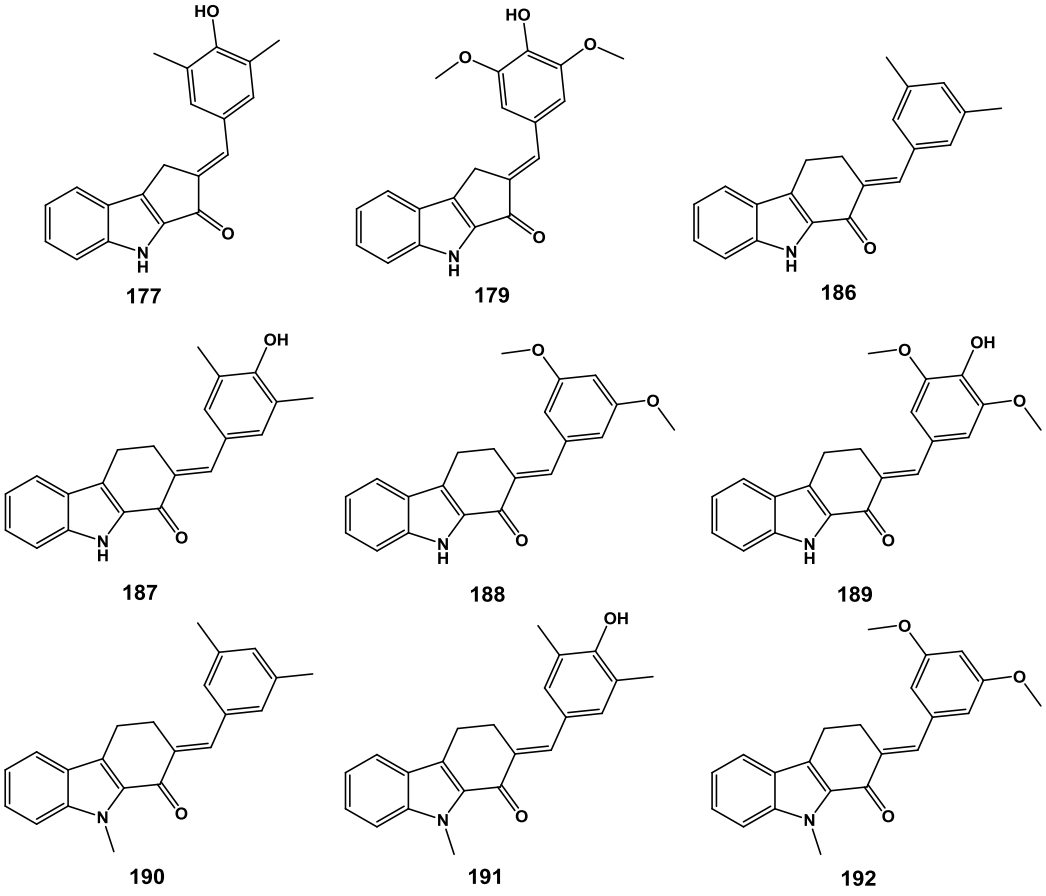


Figure 44: Compounds selected for one-dose testing

Nine diverse compounds were selected from the library of compounds synthesised. Compounds **177**, **179**, **186**, **187**, **188**, **189**, **190**, **191** and **192** were chosen and the NCI accepted to test all nine compounds (Figure 44). These compounds were chosen as they incorporate all of the structural differences explored within the analogues.

The results from one-dose data are presented as a mean graph of the percentage growth of the cancer cells. The growth is measured relative to the control which is not treated with the drug and relative to the number of cells at time zero. The mean graph displays growth inhibition (values between 0 and 100) and lethality (values less than 0). A value of 100 means no growth inhibition and a value of 60 would mean 40% growth inhibition. A value of 0 means no net growth over the assay and a value of -100 would mean all cells are dead.

Good growth inhibition is important; however, selectivity is also of interest when analysing the data. Table 14 displays the mean growth inhibition of 9 tested analogues (see appendices **8.4.2.** for mean graphs).

Compound	**Mean Growth Inhibition Value**	**Growth Inhibition (%)**	**Range**	**Selected for five-dose testing?**
177	32.85	67.15 %	123.79	Selected
179	95.04	4.96 %	68.41	Not selected
186	26.76	73.24 %	112.94	Selected
187	22.14	77.86 %	124.36	Selected
188	56.97	43.03 %	102.08	Selected
189	88.06	11.94 %	79.21	Not selected
190	79.22	20.78 %	75.74	Not selected
191	34.20	65.80 %	103.60	Selected
192	90.22	9.78 %	40.08	Not selected

Table 14: Mean growth inhibition of one-dose data

Compound **187** has the best mean growth inhibition of 77.86 % with the greatest range of 124.36. This compound contains the free indole, a phenol and the six membered cyclic ketone and has been selected for the five-dose screen. Compounds **177** and **186** both have

promising mean growth inhibitions of 67.15 % and 73.24 % respectively. These both also have a high range which indicates the possibility of better selectivity and were selected for five-dose testing.

Analogue **188** does not contain the phenol and has the lowest growth inhibition (43.03 %) of the compounds selected. It does have a good range of 102.08 so is therefore also of interest.

5.6.2. Five-Dose Data

Parameters which are collected in the five-dose screen include the GI_{50} value, the TGI and the LC_{50}. The GI_{50} value measures 50 % inhibition of cell growth and indicates the concentration needed to reduce the growth of treated cells to half that of untreated cells. The TGI (100 %, total growth inhibition) is the concentration required to inhibit cell growth completely. Finally, the LC_{50} is the lethal concentration which is the concentration required that kills treated cells by 50 %. Table 15 displays the results from the 5 analogues selected for five-dose data (see appendices **8.4.3.** for mean graphs).

Compound	**Growth Inhibition from one-dose data (%)**	**Mean GI_{50} (μM)**	**Range**	**Mean TGI (μM)**	**Mean LC_{50} (μM)**	**Selected for repeat of five-dose testing?**
177	67.15 %	1.29	162	39.8	87.1	Selected
186	73.24 %	1.41	93	40.7	89.1	Not selected
187	77.86 %	0.62	178	11.5	44.7	Selected
188	43.03 %	6.79	13.8	58.9	97.7	Not selected
191	65.80 %	0.91	151	16.6	72.4	Selected

Table 15: GI_{50} value, mean TGI and mean LC_{50} from five-dose data of five tested analogues

Compound **188** is the least active of the five analogues tested with a GI_{50} value of 6.79 μM and a low range value of only 13.8. It also requires the highest concentration of 58.9 μM in order to inhibit growth by 100 %. Analogue **188** also had the lowest growth inhibiton of 43.03 % from the one-dose data and was not selected for a repeat of the five-dose screen.

Compound **187** displayed the most potential in the one-dose data and is again the most active of the five analogues tested in the five-dose screen with a GI_{50} vaue of 0.62 μM. This compound also has the lowest concentration required to inhibit cell growth completely with a TGI value of 11.5 μM and was selected for a repeat of the five-dose screen.

Analogues **177** and **191** both display good growth inhibition with GI_{50} values of 1.29 μM and 0.91 μM respectively and also have high range values of 162 and 151. Analogue **177** is a 5-membered cyclic ketone and **191** is a 6-membered cyclic ketone, but both compounds contain the free phenol and methyl groups as the hydrophobic region.

Surprisingly, analogue **186** which showed strong initial growth inhibition in the one-dose data (73.24 %) has not been selected for a repeat of five-dose data. This analogue has relatively poor activity compared to the other analogues with a GI_{50} value of 1.41 μM.

Most of these analogues also display a subtle trend. The GI_{50} values are greater than the mean GI_{50} value across most of the melanoma cell lines tested. Figure 45 displays this trend for compound **187**.

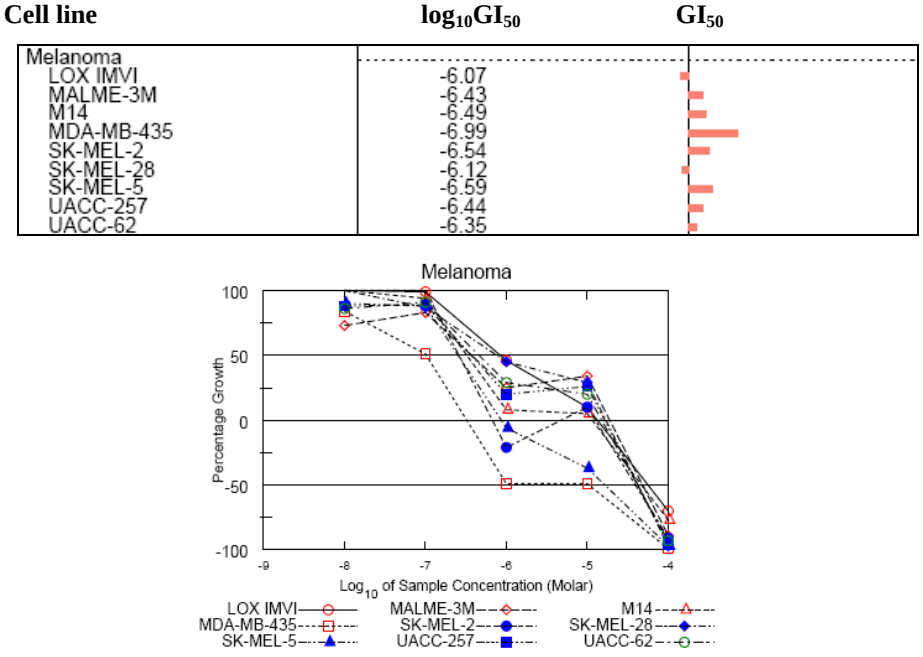


Figure 45: GI_{50} values in melanoma cell lines for analogue **187**

Compounds **177** (Figure 46) and **191** (Figure 47) were the other two analogues selected for repeat five-dose testing and these also exhibit the same trend. The GI_{50} values are greater than the mean GI_{50} value across most of the melanoma cell lines.

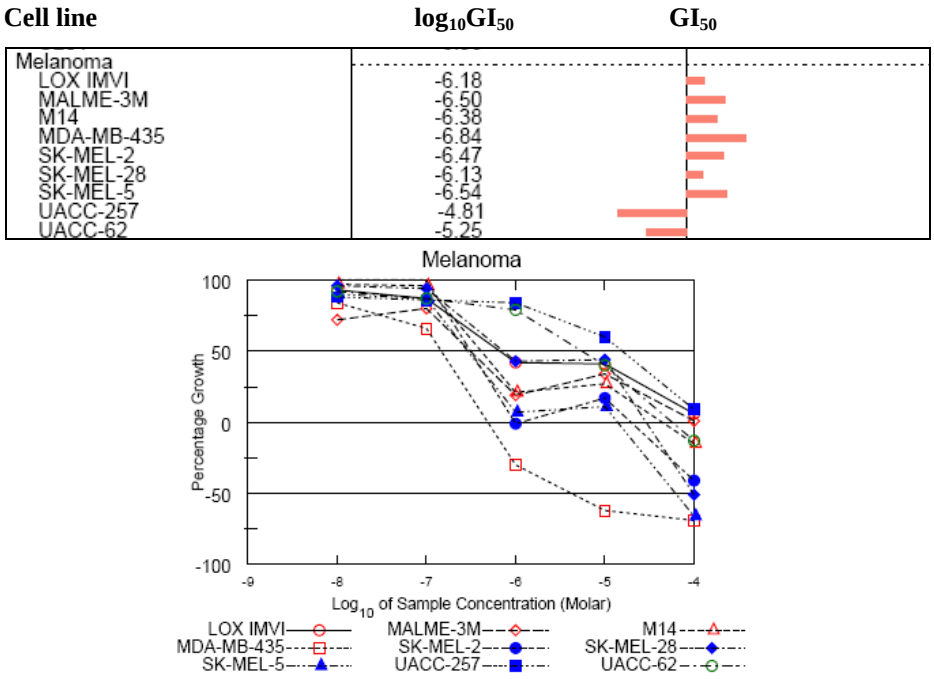


Figure 46: GI_{50} values in melanoma cell lines for analogue **177**

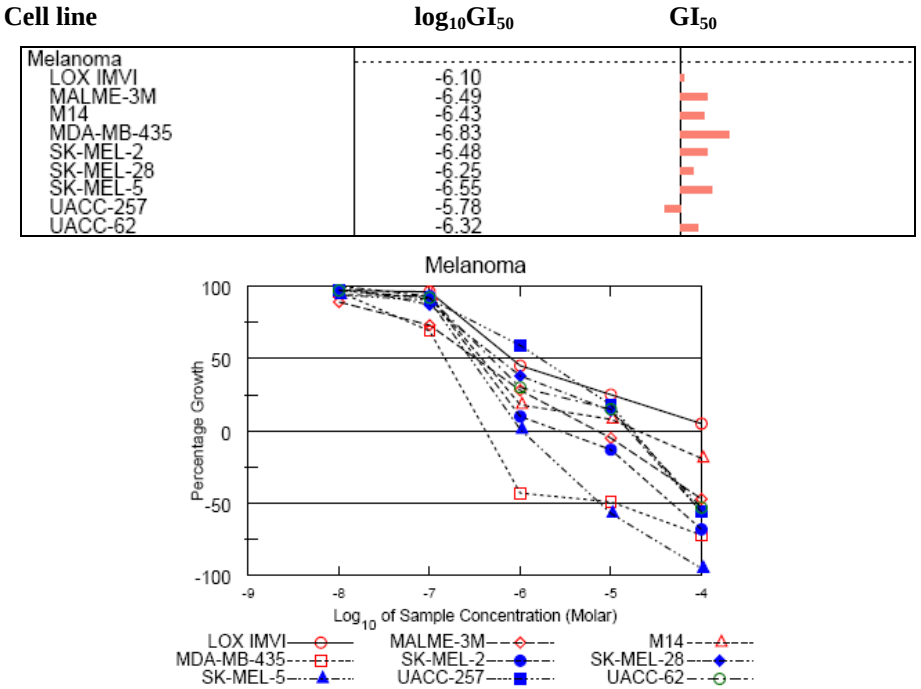


Figure 47: GI_{50} values in melanoma cell lines for analogue **191**

5.6.3. Repeat of Five-Dose Data

Interestingly all three lead compounds which were selected for a repeat of five-dose testing contain the same 4-hydroxy-3,5-dimethylphenyl moiety in their structure (Figure 48). They do differ slightly in the other regions of their structure with one indole protected with a methyl group and another containing a 5-membered cyclic ketone.

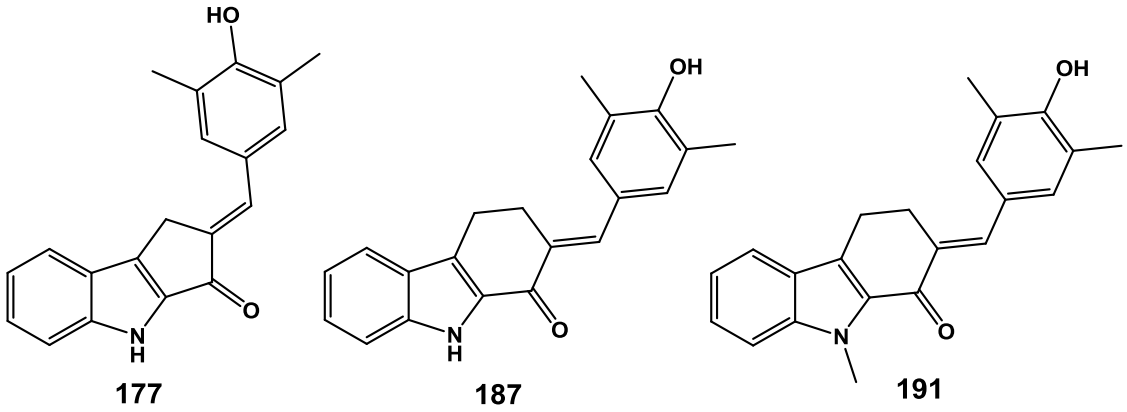


Figure 48: Three lead compounds identified from five-dose data

Table 16 outlines the results from the repeat of the five-dose screen for the three lead compounds **177**, **187** and **191**.

Compound	**Growth Inhibition from one-dose data (%)**	**Mean GI_{50} from initial five-dose data (µM)**	**Mean GI_{50} (µM)**	**Range**	**Mean TGI (µM)**	**Mean LC_{50} (µM)**
177	67.15 %	1.29	1.35	200	34.7	95.5
187	77.86 %	0.62	0.57	91	8.71	43.7
191	65.80 %	0.91	0.97	186	19.5	79.4

Table 16: Results from the repeat of five-dose data

Analogue **177** is the least active out of the three compounds tested with a mean GI_{50} value of 1.35 µM and the range for this compound is a maximum value of 200.

Compound **187** is again the most active of the three analogues tested with a mean GI_{50} value of 0.57 μM. However, in this second screen, the range for this compound is much lower than the previous results with a value of 91. Compound **187** also has the lowest concentration required to inhibit cell growth by 100 % with a TGI value of 8.71 μM.

Compounds **187** and **191** were not selected by the NCI for further testing. Compound **177** however has been selected for Biological Evaluation Committee (BEC) review (Figure 49). Members of this committee review data for compounds and decide whether to select them for additional studies. The review is based on criteria, including drugability, how novel the structure is, its mechanism of action and its potency. If the analogue is selected then more compound will be submitted to the NCI for further testing. Analogue **177** was therefore the compound selected for COMPARE analysis.

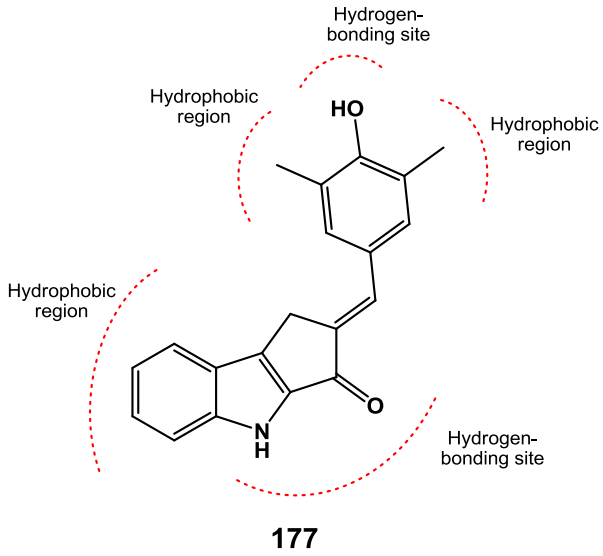


Figure 49: Lead compound **177** identified from five-dose data

5.6.4. COMPARE Analysis

The National Cancer Institute COMPARE programme utilises the activity profile created in the mean graph data from the five-dose data and evaluates this to find other compounds within the NCI database with similar activity patterns. If the activity pattern of the 'seed' compound is similar to a known active compound, then it is likely that the two compounds will have similar targets and mechanisms of action. This programme can potentially assign a

suggested mechanism of action for novel compounds simply by comparison of activity profiles across 60 cancer cell lines.

Compound **177** was selected for COMPARE analysis and an average from both sets of 5 dose data was used in the Public Standard Agents database to give more reliable reports using GI_{50}, TGI and LC_{50}.

Table 17 displays the results from the COMPARE analysis using GI_{50} data (see appendices **8.4.5** for full table). The activity profile of analogue **177** was analysed against the 'standard agents public database' and a range of microtubule binders were amongst the top ranked compounds. The percentage correlations of the chosen tubulin binders displayed in Table 17 range from 29 % up to 38 %. The correlation values are relatively low, so no firm conclusions can be made, however a wide range of tubulin binders were associated with analogue **177**. The association with a number of tubulin binders may indicate analogue **177** has the same mode of action.

Rank	Database	Seed compound	Target compound	Correlation (%)	Mecanism of action?
1	Standard Agents	**177**	**Maytansine**	38	**Tubulin binder** - inhibits microtubule assembly
4	Standard Agents	**177**	**Rhizoxin**	34	**Tubulin binder** - inhibits microtubule assembly
5	Standard Agents	**177**	**Vinblastine sulphate**	34	**Tubulin binder -** inhibits microtubule assembly
11	Standard Agents	**177**	**Cisplatin**	30	Crosslinks DNA interfering with cell division by mitosis
17	Standard Agents	**177**	**Paclitaxel**	29	**Tubulin binder** - inhibits microtubule assembly

Table 17: COMPARE analysis in the standard agents database of compound **177** using GI_{50} values

The same tubulin-binding compounds were also amongst the top-ranked compounds which match the TGI and LC_{50} activity profiles of compound **177** (Table 18 and Table 19).

Rank	Database	Seed compound	Target compound	Correlation (%)	Mecanism of action?
5	PUBLIC TGI	**177**	**Paclitaxel**	33	**Tubulin binder** - interferes with the breakdown of microtubules
9	PUBLIC TGI	**177**	**Maytansine**	29	**Tubulin binder** - inhibits microtubule assembly
11	PUBLIC TGI	**177**	**Vinblastine sulphate**	27	**Tubulin binder -** inhibits microtubule assembly

Table 18: COMPARE analysis in the standard agents database of compound **177** using TGI values

Rank	Database	Seed compound	Target compound	Correlation (%)	Mecanism of action?
2	PUBLIC LC_{50}	**177**	**Maytansine**	43	**Tubulin binder** - inhibits microtubule assembly
4	PUBLIC LC_{50}	**177**	**Paclitaxel**	27	**Tubulin binder** - interferes with the breakdown of microtubules

Table 19: COMPARE analysis in the standard agents database of compound **177** using LC_{50} values

In this chapter is reported a range of novel indanocine analogues along with their anticancer activitities. One lead compound **177** has been identified as a promising anticancer agent and is under BEC review by the NCI. COMPARE data indicate that compound **177** possibly targets the microtubules and inhibits microtubule assembly. This mode of action is consistent with indanocine **76**, which is the original analogue that these libraries were based on. Further tests to verify this could be performed to strengthen this hypothesis.

5.7. Conclusions

The work in this chapter describes the attempted synthesis of a range of indanocine analogues. Indanocine is an example of a biologically active compound which contains the indanone moiety within its structure. The aim of this work was to synthesise indole containing analogues with spatial similarities to indanocine and to test them for biological activity.

The proposed synthetic route contained two steps, the first step was a Friedel-Craft acylation reaction and the second step was a Claisen-Schmidt condensation reaction. This synthetic route was used to produce fifteen analogues in mid to low yield under varying conditions. Pure compounds were isolated and tested for preliminary biological activity against two cancer cell lines; HT29 and MDA231. A wide variety of biological activities were observed with three analogues giving promising results; **186**, **187** and **188**. These three compounds individually resulted in IC_{50} values <7 μM against the HT29 cancer cell line and analogue **186** gave an IC_{50} value of 5.4 μM against the MDA231 cancer cell line.

Nine of the fifteen isolated compounds, with a variety of structural motifs, were accepted for testing by the NCI. The analogues were initially tested using a single dose in the NCI 60 cell line screen. Five analogues were selected for further testing in a five-dose screen based on their high growth inhibition results in one-dose testing. Analogues **187**, **191** and **177** showed promising results with mean GI_{50} vaues of 0.62, 0.91 and 1.29 μM respectively. These were selected for repeat five-dose testing, after which compound **177** was selected for review by the NCI Biological Evaluation Committee.

6. Conclusions

The primary aim of this thesis was to develop new syntheses for a range of analogues that contain the tetrahydroisoquinoline or dihydroisoquinolinone framework, and to explore their biological activities.

Synthesis of the tetrahydroisoquinoline framework was initially investigated using a Friedel-Crafts alkylation reaction. Although the appropriate precursors were synthesised in good yields, the Friedel-Crafts alkylation approach proved unsuccessful. During attempted cyclisation reactions an unexpected, but interesting, carbonate side product was observed. The carbonate side product was formed by incorporation of CO_2 from the atmosphere in the presence of Ag_2O. This was further explored as a potential synthetic route to carbonate compounds and a range of carbonates were subsequently synthesised in high yields. Alternative, more favourable, Friedel-Crafts acylation reaction conditions were then explored. This approach was also unsuccessful however, the use of $SOCl_2$ led to an interesting side product with both a sulfur and chlorine atom being incorporated into the novel compound **69**.

The synthesis of the dihydroisoquinolinone framework was investigated. An initial proposed synthetic route involved the synthesis of the dihydroisoquinolinone framework *via* the corresponding indanone. The formation of A/B indanones was acheived *via* an intramolecular Friedel-Crafts acylation reaction using TFAA as an activator, and a library of indanones were synthesised in high yield. A Schmidt reaction was used to synthesise a library of A/B lactams from their corresponding indanones in good yield. The synthesis of A/B/C indanones was achieved using similar reaction conditions to those for A/B indanones, however the synthesis was sensitive to temperature and reaction time. A single A/B/C lactam **148** was reliably synthesised *via* a Schmidt reaction.

Due to the capricious nature of the intial synthetic route, an alternative synthesis was explored to access the A/B/C dihydroisoquinolinone framework. The first step was a Heck cross-coupling reaction and the second step was a modified Curtius rearrangement. This route was used to synthesis A/B/C lactam **170** in an overall 51 % yield, which gave an IC_{50}

value of 9 μM when tested against the HT29 colon cancer cell line. Lactam **170** lacks functionality on the C-ring; if functionality is incorporated then the biological activity should further improve.

Indanocine is an example of a biologically active compound which contains an indanone moiety within its structure. The synthesis of novel analogues of indanocine was explored. The proposed synthetic route involved two steps, the first step was a Friedel-Craft acylation reaction and the second step was a Claisen-Schmidt condensation reaction. This synthetic route was used to produce fifteen analogues in mid to low yield under varying conditions. Compounds **186** and **187** when tested against cancer cell line HT29 resulted in IC_{50} values <7 μM and analogue **186** gave an IC_{50} value of 5.4 μM against the MDA231 cancer cell line.

Nine of the fifteen isolated compounds, with a variety of structural motifs, were accepted for testing by the NCI. The analogues were initially tested using a single dose in the NCI 60 cell line screen. Five analogues were selected for further testing in a five-dose screen based on their high growth inhibition results in one-dose testing. Analogues **187**, **191** and **177** showed promising results with mean GI_{50} values, across 60 cell lines, of 0.62, 0.91 and 1.29 μM respectively. These were selected for repeat five-dose testing, after which compound **177** was selected for review by the NCI Biological Evaluation Committee. Compound **177** is currently under consideration by the NCI as a candidate for further testing.

The work undertaken in this thesis has pioneered new synthetic routes that can be used to produce biologically active compounds. The synthetic chemistry within this thesis was conducted using a framework approach. The biologically active compounds synthesised represent only a portion of the compounds that could be accessed *via* the routes that were developed, and further work could potentially unlock many interesting medicinal candidates.

7. Experimental

7.1. General Experimental

Chemicals, solvents and reagents used are commercially available and were used without further purification.

All TLC's were carried out on Merck Aluminium-backed TLC plates Silicagel 60 F254. They were viewed using UV light of wavelength 254 nm and then stained with either potassium permanganate or 2,4-dinitrophenylhydrazine (DNP), which were then heated gently with a heatgun. Column chromatography was used to purify compounds with Merck Silica gel (0.040-0.063 mm). Compounds were loaded as a $CHCl_3$ solution or dry loaded by adsorption onto silica.

^{1}H NMR spectra were obtained on JEOL Eclipse (270 MHz), Varian Mercury VX (400 MHz) or Bruker Avance III (400 MHz) spectrometers. ^{13}C NMR spectra were obtained on JEOL Eclipse (67.9 MHz), Varian Mercury VX (100 MHz) or Bruker Avance III (100 MHz) spectrometers. The chemical shifts are recorded in parts per million (ppm) with reference to residual solvent peaks. The coupling constants *J* are in Hertz (Hz), recorded to the nearest 0.5 Hz and are not rationalised. The multiplicities are assigned as a singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint), septet (sept), doublet of doublets (dd), doublet of triplets (dt), triplet of doublets (td), doublet of doublet of doublets (ddd), broad singlet (brs) and multiplet (m). The +ve or -ve symbols after the carbon NMR peaks represent the number of protons attached where +ve indicates CH and CH_3 and –ve indicates C and CH_2. ^{19}F NMR spectra were obtained on a Bruker Avance III (100 MHz) spectrometer.

Mass Spectrometry (MS) and High Resolution Mass Spectrometry (HRMS) were carried out on a micrOTOF^{TM} from Bruker Daltonics (Bremen, Germany), which uses electrospray source (ESI-TOF). It has a mass accuracy of 5 ppm externally calibrated using sodium formate solution, which is applicable to both positive and negative ionisation modes. All theoretical and calculated values are reported to 4 decimal places. In the event of an observed ion having an error of greater than 5 ppm from the theoretical value it is still reported with

the margin of error stated. The data was processed using external calibration with the Bruker Daltonics software, DataAnalysis^{TM} as part of the overall hardware control software, Compass 1.1^{TM} and the samples were analysed under positive electrospray ionisation mode. Ions are usually present as both protonated molecules and as the sodium complex. The mass spectrometer collects information on both accurate mass of the molecules and isotope patterns enabling determination of the molecular formula. The samples are added by syringe pump or flow injection using an autosampler in an Agilent 1100 LC system.

Infrared spectra (IR) were recorded on either Perkin-Elmer, Spectrum RX I FT-IR system and all values are recorded in cm^{-1}.

Melting points were obtained using a Reichert-Jung heated-stage microscope.

X-ray Crystallography was performed by Dr Mary Mahon. Single crystals were analysed at 150(2) K using graphite monochromated Mo(Kα) radiation and a Nonius Kappa CCD diffractometer. The structures were solved using SHELXS-97 and refined using SHELXL-97.

7.2. MTS Cell proliferation assay protocol

This assay uses a 96-well plate format to determine cell viability and is based on the Promega Cell Titer 96 Aqueous One Solution Cell Proliferation Assay. Seed densities of 500 cells per well were used and final drug concentrations of 500 µM, 200 µM, 100 µM, 50 µM, 20 µM, 10 µM, 5 µM, 2 µM, 1 µM and 500 nM in 1% DMSO. Drugs were incubated with the HT29 cell line for 72 hours prior to reading and IC_{50} curves were generated using SigmaPlot 8 software.

General Procedure:

1. 96-Well tissue culture plates: 100 µL per well final volume.
2. Cell suspension added in 50 µL culture medium.
   Test compound added in 50 µL culture medium (plus other solvent if necessary).
3. Cell suspension added to wells several hours before adding test compound.
   This can be from 2-3 hours, depending on the experiment.
   Cells plus test compound incubated at 37 ^{o}C, 5 % CO_2 in humidified air for 3 days.
4. MTS reagent added, 20 µL per well.
5. Plate returned to incubator for 3 hours.
6. OD_{490nm} recorded after 3 hours.

7.3. Chapter Two - 1,2,3,4-Tetrahydroisoquinolines

7.3.1. Alkylation

General Procedure 1, Synthesis of bis-alkylation of primary amines

nBu_4NI (0.3 eq.) was added to a rapidly stirred suspension of the required amine (1 eq.), the required benzyl chloride (2.2 eq.) and K_2CO_3 (3 eq.) in MeCN (6 mL/ 1 mmol) open to the air. After 3.5 hours at 82 ^oC, the mixture was concentrated under reduced pressure. The residue was suspended in H_2O (30 mL) and the product extracted with EtOAc (4 x 30 mL). The combined organic layers were washed with brine (30 mL), dried on $MgSO_4$, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) EtOAc gradient column], the desired product was isolated.

General Procedure 2, Iodination reaction open to the air

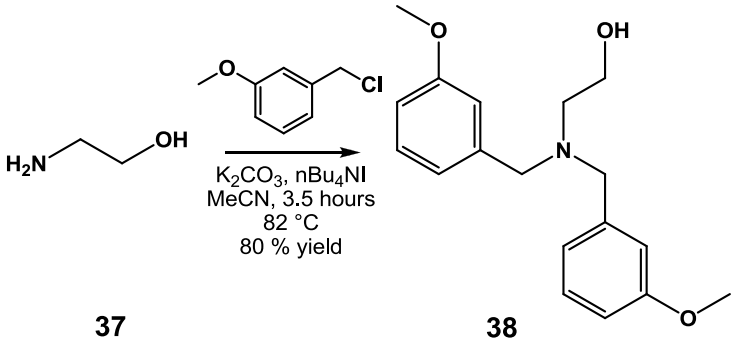
The required alkylated amino alcohol (1 eq.), I_2 (1.1 eq.), PPh_3 (1.1 eq.) and imidazole (1.1 eq.) were suspended in CH_2Cl_2 (4 mL/ 1 mmol) open to the air. After 3.5 hours of stirring at room temperature, the mixture was concentrated under reduced pressure. EtOAc (30 mL) and sat. aq. $NaHCO_3$ solution (40 mL) were added, the layers separated and the aqueous layer extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with brine (30 mL), dried on $MgSO_4$, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], the desired product was isolated.

General Procedure 3, Iodination reaction under N_2

The required alkylated amino alcohol (1 eq.), I_2 (1.1 eq.), PPh_3 (1.1 eq.) and imidazole (1.1 eq.) were suspended in CH_2Cl_2 (4 mL/ 1 mmol) under a N_2 atmosphere. After 5 hours of stirring at room temperature, the mixture was concentrated under reduced pressure. EtOAc (30 mL) and sat. aq. $NaHCO_3$ solution (40 mL) were added, the layers separated and the aqueous layer extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with brine (30 mL), dried on $MgSO_4$, filtered and concentrated under reduced pressure. After

column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the desired product was isolated.

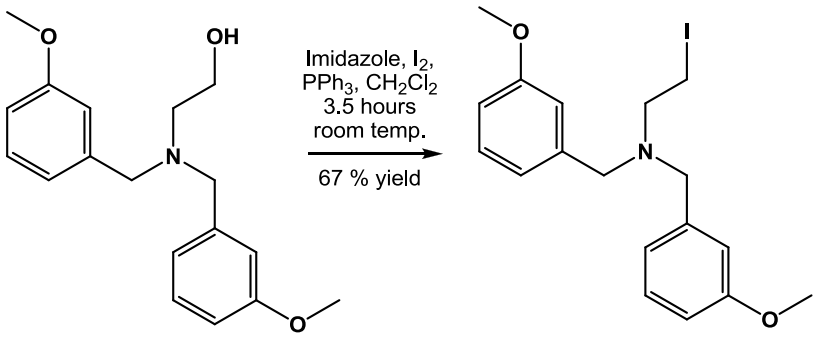
Synthesis of 2-(bis(3-methoxybenzyl)amino)ethanol 38



37 **38**

According to **General Procedure 1**, using ethanolamine **37** (302 μL, 5 mmol, 1 eq.), *n*Bu_4NI (554 mg, 1.5 mmol, 0.3 eq.), 3-methoxybenzyl chloride (1.6 mL, 11 mmol, 2.2 eq.) and K_2CO_3 (2.07 g, 15 mmol, 3 eq.) in MeCN (30 mL), alcohol **38** (1.2 g, 80 % yield) was isolated as a colourless oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.24 (2H, t, *J* = 8.0 Hz, ArC(5)*H*), 6.91 (2H, d, *J* = 7.5 Hz, ArC(4)*H*), 6.87 (2H, t, *J* = 1.5 Hz, ArC(2)*H*), 6.79 (2H, dd, *J* = 8.0, 1.5 Hz, ArC(6)*H*), 3.80 (6H, s, ArOCH_3), 3.60 (4H, s, ArCH_2NCH_2Ar), 3.58 (2H, t, *J* = 5.0 Hz, NCH_2CH_2OH) and 2.67 (2H, t, *J* = 5.0 Hz, NCH_2CH_2OH). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 159.7- (Ar*C*(1)), 140.4- (Ar*C*(3)), 129.4+ (Ar*C*(5)H), 121.2+ (Ar*C*(2)H), 114.6+ (Ar*C*(4)H), 112.4+ (ArC(6)H), 58.6- (NCH_2CH_2OH), 58.2- (ArCH_2NCH_2Ar), 55.1+ (ArOCH_3) and 54.9- (NCH_2CH_2OH). **MS** *m/z* (+ESI) 302 (100 %, MH^+) and 324 (13 %, MNa^+). **HRMS** (+ESI) Found MH^+ 302.1748, $C_{18}H_{24}NO_3$ requires 302.1756 and found MNa^+ 324.1554, $C_{18}H_{23}NNaO_3$ requires 324.1576. **IR** ν_{max}(liquid film): 3451 (OH), 2943 (C-H), 1600 (C=C) and 1049 (C-O). **Rf** (50 % EtOAc in light petroleum (b.p. 40-60 ^{o}C) 0.6.

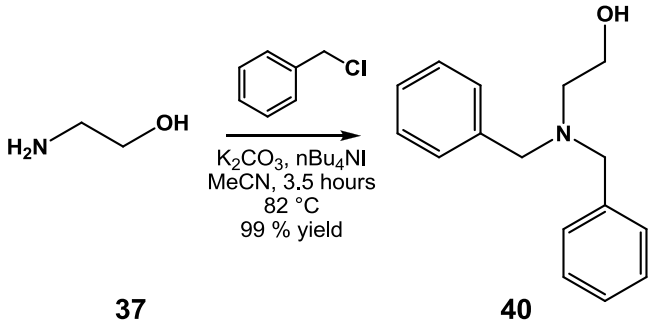
Synthesis of 2-iodo-*N*,*N*-bis(3-methoxybenzyl)ethanamine 39



38 **39**

According to **General Procedure 2**, using alcohol **38** (602 mg, 2 mmol, 1 eq.), I_2 (558 mg, 2.2 mmol, 1.1 eq.), PPh_3 (577 mg, 2.2 mmol, 1.1 eq.) and imidazole (150 mg, 2.2 mmol, 1.1 eq.) in CH_2Cl_2 (8 mL) iodide **39** (550 mg, 67 % yield) was isolated as a clear brown oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.22 (2H, t, *J* = 8.0 Hz, ArC(5)*H*), 7.01 (2H, s, ArC(2)*H*), 6.95 (2H, d, *J* = 7.5 Hz, ArC(6)*H*), 6.79 (2H, dd, *J* = 8.0 and 2.5 Hz, ArC(4)*H*), 3.82 (6H, s, ArOCH_3), 3.60 (4H, s, ArCH_2NCH_2Ar), 3.19 (2H, t, *J* = 7.5 Hz, NCH_2CH_2I) and 2.82 (2H, t, *J* = 7.5 Hz, NCH_2CH_2I). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 159.7- (Ar*C*(1)), 140.7 (Ar*C*(3)), 129.2+ (Ar*C*(5)H), 121.0+ (Ar*C*(2)H), 114.2 (Ar*C*(4)H), 112.6+ (Ar*C*(6)H), 58.0- ($ArCH_2NCH_2Ar$), 55.9- (NCH_2CH_2I), 55.2+ ($ArOCH_3$) and 4.30- (NCH_2CH_2I). **MS** *m/z* (+ESI) 412 (MH^+) and 284 (58 %, aziridinium$^+$). **HRMS** (+ESI) Found aziridinium$^+$ 284.1646, $C_{18}H_{22}NO_2$ requires 284.1651. **IR** ν_{max}(liquid film): 2939 (C-H), 1600 (C=C) and 1050 (C-O). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^oC) 0.8.

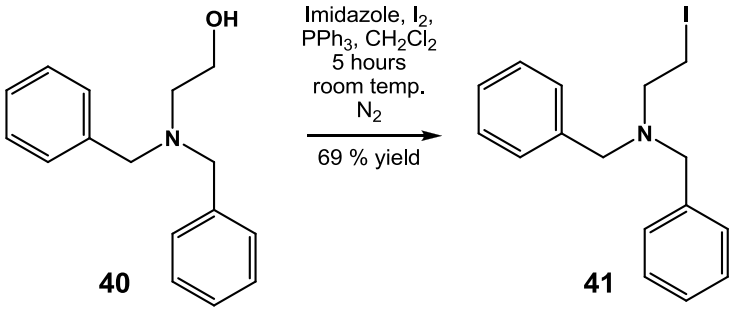
Synthesis of 2-(dibenzylamino)ethanol 40



37 **40**

According to **General Procedure 1**, using ethanolamine **37** (600 μL, 10 mmol, 1 eq.), *n*Bu_4NI (1.11 mg, 3 mmol, 0.3 eq.), benzyl chloride (2.5 mL, 22 mmol, 2.2 eq.) and K_2CO_3 (4.15 g, 30 mmol, 3 eq.) in MeCN (30 mL) alcohol **40** (2.4 g, 99 % yield) was isolated as a white solid, **m.p.** 42-44 ^oC [lit.[161] 38 ^oC]. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.35-7.26 (10H, m, ArC*H*), 3.64 (4H, s, Ph*CH*$_2$N*CH*$_2$Ph), 3.59 (2H, t, *J* = 5.5 Hz, NCH_2CH_2OH) and 2.68 (2H, t, *J* = 5.5 Hz, NCH_2CH_2OH). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 138.7- (Ar*C*(1)), 129.0+ (Ar*C*(3)H), 128.4+ (Ar*C*(2)H), 127.2+ (Ar*C*(4)H), 58.5- (NCH_2CH_2OH), 58.2- ($PhCH_2NCH_2Ph$) and 54.8- (NCH_2CH_2OH). **MS** *m/z* (+ESI) 242 (100 %, MH^+) and 264 (1 %, MNa^+). **HRMS** (+ESI) Found MH^+ 242.1546, $C_{16}H_{20}NO$ requires 242.1545 and found MNa^+ 264.1368, $C_{16}H_{19}NNaO$ requires 264.1364. **IR** ν_{max}(liquid film): 3321 (OH) and 1600 (C=C). **Rf** (20 % EtOAc in light petroleum (b.p. 40-60 ^oC) 0.3. Spectroscopic data are consistent with those previously reported by Wurm *et al.*[161]

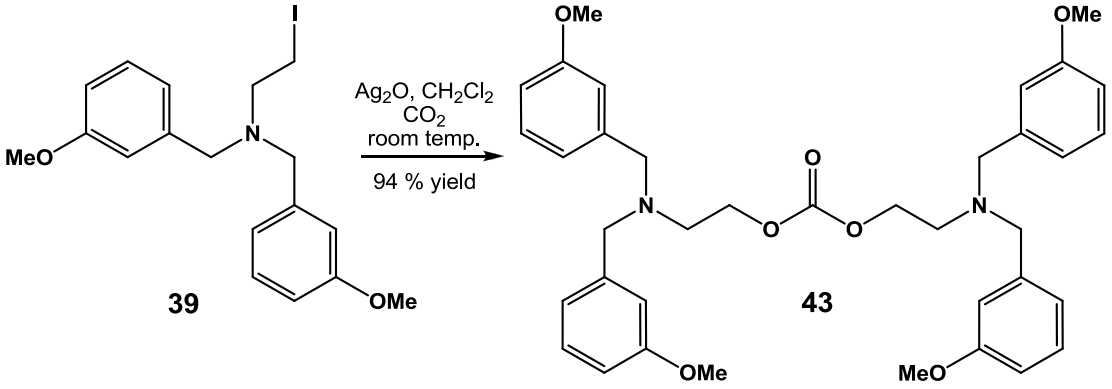
Synthesis of *N,N*-dibenzyl-2-iodoethanamine 41



40 **41**

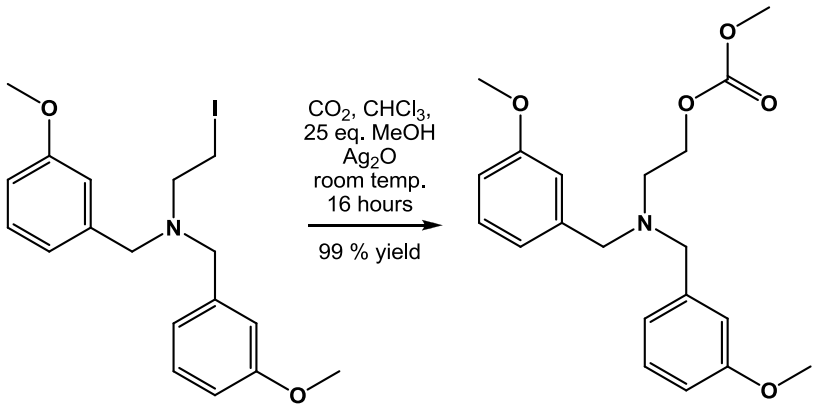
According to **General Procedure 2**, using alcohol **40** (743 mg, 3.08 mmol, 1 eq.), I_2 (858 mg, 3.38 mmol, 1.1 eq.), PPh_3 (887 mg, 3.38 mmol, 1.1 eq.) and imidazole (230 mg, 3.38 mmol, 1.1 eq.) in CH_2Cl_2 (20 mL), iodide **41** (744 mg, 69 % yield) was isolated as a clear brown oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.31 (4H, t, *J* = 7.5 Hz, ArC(3)*H*), 7.27-7.23 (4H, m, ArC(2)*H*), 7.19-7.15 (2H, m, ArC(4)*H*), 3.56 (4H, s, Ph*C*H_2N*C*H_2Ph), 3.09 (2H, t, *J* = 7.5 Hz, NCH$_2$C*H*$_2$I) and 2.76 (2H, t, *J* = 7.5 Hz, NC*H*$_2$CH$_2$I). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 139.0- (Ar*C*(1)), 128.8+ (Ar*C*(3)H), 128.3+ (Ar*C*(2)H), 127.1+ (Ar*C*(4)H), 58.1- (Ph*C*H$_2$N*C*H$_2$Ph), 56.1- (N*C*H$_2$CH$_2$I) and 4.0- (NCH$_2$*C*H$_2$I). **MS** *m/z* (+ESI) 224 (100 %, M^+-I) and 352 (11 %, MH^+). **HRMS** (+ESI) Found MH^+ 352.0561, $C_{16}H_{19}IN$ requires 352.0562. **IR** ν_{max}(liquid film): 2925 (CH) and 1602 (C=C). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C) 0.8.

Synthesis of Carbonic acid, bis(N,N-di(3-methoxybenzyl)aminoethyl) carbonate 43



Iodide **39** (133 mg, 0.32 mmol, 1 eq.) was dissolved in CH_2Cl_2 (2 mL) and stirred under an atmosphere of CO_2 (balloon) at room temperature for 2 hours. Ag_2O (81 mg, 0.35 mmol, 1.1 eq.) was added and the mixture was stirred under an atmosphere of CO_2 at room temperature for 16 hours. The mixture was filtered through a pad of celite, the celite was washed with CH_2Cl_2 and the reaction mixture was concentrated under reduced pressure to afford symmetrical carbonate **43** (95 mg, 94 % yield) as a clear yellow oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.20 (4H, t, *J* = 8.0 Hz, ArC(5)*H*), 6.95 (4H, s, ArC(2)*H*), 6.93 (4H, d, *J* = 8.0 Hz, ArC(4)*H*), 6.77 (4H, dd, *J* = 8.0 and 2.0 Hz, ArC(6)*H*), 4.19 (4H, t, *J* = 6.5 Hz, NCH_2CH_2O), 3.78 (12H, s, $ArOCH_3$), 3.63 (8H, s, $ArCH_2NCH_2Ar$) and 2.76 (4H, t, *J* = 6.5 Hz, NCH_2CH_2O). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 159.7- (Ar*C*(3)) 155.1- (*C*=O), 140.9- (Ar*C*(1)), 129.2+ (Ar*C*(5)H, 121.0+ (Ar*C*(4)H), 114.0+ (Ar*C*(2)H), 112.5+ (Ar*C*(6)H), 65.8- (NCH_2CH_2O), 58.6- ($ArCH_2NCH_2N$), 55.1+ ($ArOCH_3$) and 51.6- (NCH_2CH_2O). **MS** *m/z* (+ESI) 651 (100 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 651.3028, $C_{37}H_{44}N_2NaO_7$ requires *MNa* 651.3004. **IR** ν_{max}(liquid film): 3054 (CH), 1744 (C=O), 1601 (C=C) and 1264 (C-O). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.4.

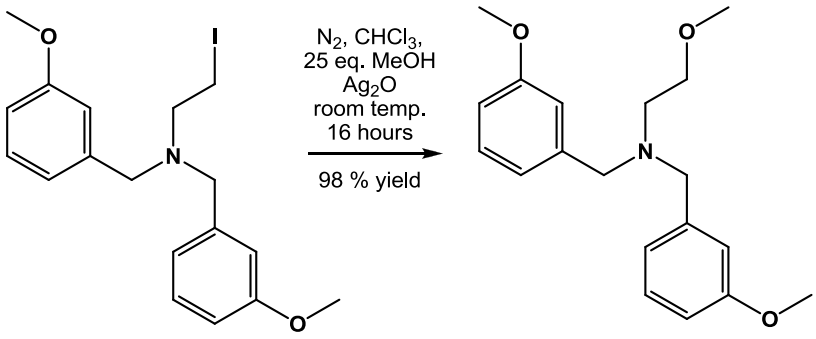
Synthesis of 2-(bis(3-methoxybenzyl)amino)ethyl methyl carbonate 44



39 **44**

Iodide intermediate **39** (44 mg, 0.11 mmol, 1 eq.) was dissolved in $CHCl_3$ (2 mL) and CO_2 (balloon) was bubbled through. MeOH (0.1 mL, 2.75 mmol, 25 eq.) was added, followed by Ag_2O (27 mg, 0.12 mmol, 1.1 eq.). The reaction mixture was stirred under an atmosphere of CO_2 at room temperature for 16 hours. The reaction was filtered through a cotton wool plug, the cotton wool was washed with $CHCl_3$ (10 mL) and the solvent was removed under reduced pressure. Traces of silver were still present so the product was dissolved in $CHCl_3$, the solution was filtered for a second time through a silica plug and the silica was washed with EtOAc (10 mL). The solvent was removed under reduced pressure to collect carbonate **44** (38 mg, 99 % yield) as a pale yellow oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_C 7.14 (2H, t, *J* = 8.0 Hz, ArC(4)*H*), 6.89 (2H, s, ArC(2)*H*), 6.86 (2H, d, *J* = 7.5 Hz, ArC(5)*H*), 6.71 (2H, dd, *J* = 8.0 and 2.5 Hz, ArC(6)*H*), 4.15 (2H, t, *J* = 6.0 Hz, NCH_2CH_2O), 3.73 (6H, s, $ArOCH_3$), 3.68 (3H, s, $(CO)OCH_3$), 3.56 (4H, s, $ArCH_2NCH_2Ar$) and 2.70 (2H, t, *J* = 6.0 Hz, NCH_2CH_2O). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 159.7- (Ar*C*(1)), 155.7- (*C*=O), 140.9- (Ar*C*(3)), 129.2+ (Ar*C*(4)H), 121.0+ (Ar*C*(2)H), 114.0+ (Ar*C*(5)H), 112.5+ (Ar*C*(6)H), 65.9- (NCH_2CH_2O), 58.6- ($ArCH_2NCH_2Ar$), 55.1+ ($ArOCH_3$), 54.7+ ($(CO)OCH_3$) and 51.7- (NCH_2CH_2O). **MS** *m/z* (+ESI) 360 (100 %, MH^+). **HRMS** (+ESI) Found MH^+ 360.1805, $C_{20}H_{26}NO_5$ requires *MH* 360.1805. **IR** ν_{max}(liquid film): 2834 (CH), 1749 (C=O), 1600 (C=C) and 1047 (C-O). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^oC)) 0.6.

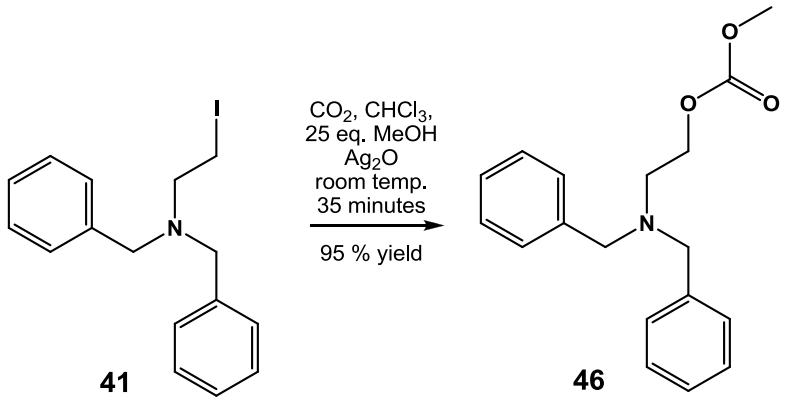
Synthesis of 2-methoxy-*N*,*N*-bis(3-methoxybenzyl)ethylamine 45



39 **45**

Iodide intermediate **39** (80 mg, 0.19 mmol, 1.0 eq.) was placed under a N_2 atmosphere for 10 minutes and then dissolved in $CHCl_3$ (3 mL). MeOH (0.2 mL, 4.75 mmol, 25 eq.) was added, followed by Ag_2O (49 mg, 0.21 mmol, 1.1 eq.) and the reaction mixture was stirred under an atmosphere of N_2 at room temperature for 16 hours. The reaction mixture was filtered through a cotton wool plug, the plug was washed with $CHCl_3$ (10 mL) and the solvent was removed under reduced pressure. Traces of silver were still present so the product was dissolved in $CHCl_3$, the solution was filtered for a second time through a silica plug and the silica was washed with EtOAc (10 mL). The solvent was removed under reduced pressure to collect ether **45** (60 mg, 98 % yield) as a pale yellow oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.21 (2H, t, *J* = 8.0 Hz, ArC(5)*H*), 6.98 (2H, d, *J* = 2.5 Hz, ArC(2)*H*), 6.95 (2H, d, *J* = 8.0 Hz, ArC(4)*H*), 6.78 (2H, dd, *J* = 8.0 and 2.5 Hz, ArC(6)*H*), 3.81 (6H, s, ArOC*H*$_3$), 3.63 (4H, s, ArC*H*$_2$NC*H*$_2$Ar), 3.50 (2H, t, *J* = 6.0 Hz, NCH$_2$C*H*$_2$O), 3.29 (3H, s, OC*H*$_3$) and 2.69 (2H, t, *J* = 6.0 Hz, NC*H*$_2$CH$_2$O). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 159.6- (Ar*C*(1)), 141.5- (Ar*C*(3)), 129.1+ (Ar*C*(5)H), 121.0+ (Ar*C*(2)H), 114.2+ (Ar*C*(4)H), 112.3+ (Ar*C*(6)H), 71.5- (NCH$_2$*C*H$_2$O), 58.8- (Ar*C*H$_2$N*C*H$_2$Ar), 58.7+ (O*C*H$_3$), 55.1+ (ArO*C*H$_3$) and 52.8- (N*C*H$_2$CH$_2$O). **MS** *m/z* (+ESI) 316 (100 %, MH$^+$) and 338 (1 %, MNa$^+$). **HRMS** (+ESI) Found MH$^+$ 316.1891, $C_{19}H_{26}NO_3$ requires *MH* 316.1907 and found MNa$^+$ 338.1718, $C_{19}H_{25}NNaO_3$ requires *MNa* 338.1727**. IR** ν_{max}(liquid film): 2833 (CH), 1600 (C=C) and 1048 (C-O). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.7.

Synthesis of 2-(dibenzylamino)ethyl methyl carbonate 46



Method A

Iodide intermediate **41** (160 mg, 0.46 mmol, 1 eq.) was dissolved in $CHCl_3$ (4 mL) and CO_2 was bubbled through. MeOH (0.09 mL, 2.30 mmol, 5 eq.) was added, followed by Ag_2CO_3 (141 mg, 0.51 mmol, 1.1 eq.). The reaction mixture was stirred under an atmosphere of CO_2 at room temperature for 3 hours. The reaction mixture was filtered through a pad of celite, the celite was washed with $CHCl_3$ (10 mL) and the solvent was removed under reduced pressure to collect carbonate **46** (127 mg, 60 % yield) as a clear pale yellow oil.

Method B

Iodide intermediate **41** (196 mg, 0.56 mmol, 1 eq.) was dissolved in $CHCl_3$ (5 mL) and CO_2 was bubbled through. MeOH (0.11 mL, 2.80 mmol, 5 eq.) was added, followed by Ag_2O (144 mg, 0.62 mmol, 1.1 eq.) and the reaction mixture was stirred under an atmosphere of CO_2 at room temperature for 3 hours. The reaction mixture was filtered through a pad of celite, the celite was washed with $CHCl_3$ (10 mL) and the solvent was removed under reduced pressure to collect carbonate **46** (158 mg, 95 % yield) as a clear pale yellow oil.

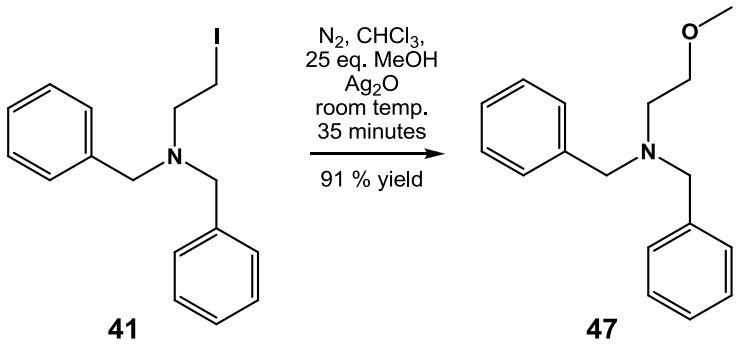
Method C

Iodide intermediate **41** (50 mg, 0.14 mmol, 1 eq.) was dissolved in $CHCl_3$ (2 mL) and CO_2 was bubbled through. MeOH (0.14 mL, 3.5 mmol, 25 eq.) was added, followed by Ag_2O (37 mg, 0.16 mmol, 1.1 eq.). The reaction mixture was stirred under an atmosphere of CO_2 at room temperature for 16 hours. The reaction mixture was filtered through a cotton wool plug, the plug was washed with $CHCl_3$ (10 mL) and the solvent was removed under reduced pressure. Traces of silver were still present so the product was dissolved in $CHCl_3$, the solution was filtered for a second time through a silica plug and the silica was washed with

EtOAc (10 mL). The solvent was removed under reduced pressure to collect carbonate **46** (40 mg, **95 % yield**) as a colourless oil.

^{1}H NMR (400 MHz; $CDCl_3$): δ_H 7.36 (4H, d, *J* = 7.0 Hz, ArC(2)*H*), 7.30 (4H, t, *J* = 7.0 Hz, ArC(3)*H*), 7.23 (2H, t, *J* = 7.0 Hz, ArC(4)*H*), 4.20 (2H, t, *J* = 6.0 Hz, NCH_2CH_2O), 3.75 (3H, s, OCH_3), 3.65 (4H, s, $PhCH_2NCH_2Ph$) and 2.76 (2H, t, *J* = 6.0 Hz, NCH_2CH_2O). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 155.7- (*C*=O), 139.2- (Ar*C*(1)), 128.7+ (Ar*C*(2)H), 128.2+ (Ar*C*(3)H), 127.0+ (Ar*C*(4)H), 66.0- (NCH_2CH_2O), 58.7- ($PhCH_2NCH_2Ph$), 54.7+ (OCH_3) and 51.6- (NCH_2CH_2O). **MS** *m/z* (+ESI) 300 (100 %, MH^+) and 322 (2 %, MNa^+). **HRMS** (+ESI) Found MH^+ 300.1597, $C_{18}H_{22}NO_3$ requires *MH* 300.1600 and found MNa^+ 322.1419, $C_{18}H_{21}NNaO_3$ requires *MNa* 322.1419. **IR** ν_{max}(liquid film): 2955 (CH), 1747 (C=O) and 1602 (C=C). **Rf** (20 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.5.

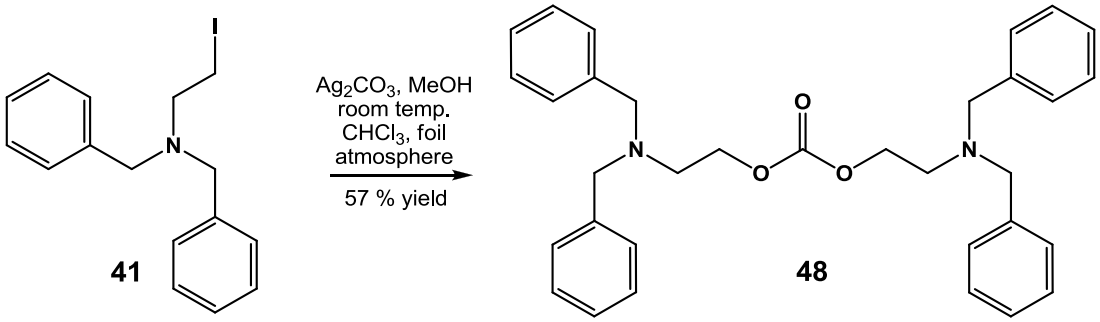
Synthesis of *N,N*-dibenzyl-2-methoxyethylamine 47



41 **47**

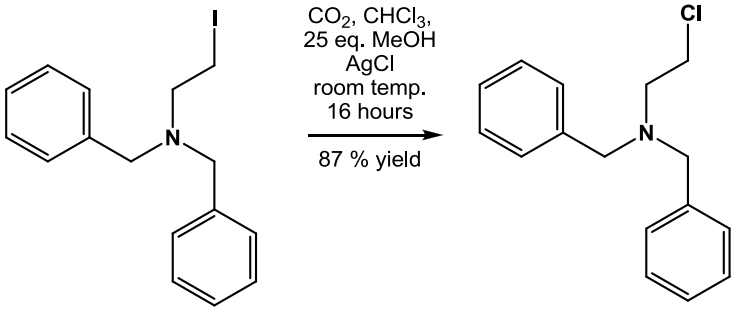
Iodide intermediate **41** (57 mg, 0.16 mmol, 1 eq.) was placed under a N_2 atmosphere for 5 minutes and then dissolved in $CHCl_3$ (2 mL). MeOH (0.16 mL, 4 mmol, 25 eq.) was added, followed by Ag_2O (41 mg, 0.18 mmol, 1.1 eq.). The reaction mixture was stirred under a N_2 atmosphere at room temperature for 35 minutes. The reaction mixture was filtered through a cotton wool plug, washed with $CHCl_3$ (10 mL) and the solvent was removed under reduced pressure. Traces of silver were still present so the product was dissolved in $CHCl_3$ and the solution was filtered for a second time through a silica plug, the silica was washed with EtOAc (10 mL) and concentrated under reduced pressure to collect ether **47** (37 mg, 91 % yield) as a colourless oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.38 (4H, d, *J* = 8.0 Hz, ArC(2)*H*), 7.30 (4H, t, *J* = 8.0 Hz, ArC(3)*H*), 7.25-7.21 (2H, m, ArC(4)*H*), 3.65 (4H, s, PhCH_2NCH_2Ph), 3.49 (2H, t, *J* = 6.5 Hz, NCH$_2$CH_2O), 3.28 (3H, s, OCH_3) and 2.66 (2H, t, *J* = 6.5 Hz, NCH_2CH$_2$O). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 139.7- (Ar*C*(1)), 128.8+ (Ar*C*(2)H), 128.2+ (Ar*C*(3)H), 126.8+ (Ar*C*(2)H), 71.5- (NCH$_2$*C*H$_2$O), 58.9- (Ph*C*H$_2$N*C*H$_2$Ph), 58.7+ (O*C*H$_3$) and 52.7- (N*C*H$_2$CH$_2$O). **MS** *m/z* (+ESI) 256 (100 %, MH$^+$). **HRMS** (+ESI) Found MH$^+$ 256.1691, $C_{17}H_{21}NO$ requires *MH* 256.1701. **IR** ν_{max}(liquid film): 2923 (CH) and 1601 (C=C). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.5. Spectroscopic data consistent with those reported by Henkel *et al*.[162]

Synthesis of bis(2-(dibenzylamino)ethyl) carbonate 48



Following a procedure reported by Teranishi[68] Ag_2CO_3 (938 mg, 3.40 mmol, 5 eq.) and MeOH (0.14 mL, 3.40 mmol, 5 eq.) were added to a stirred solution of iodide intermediate **41** (237 mg, 0.68 mmol, 1 eq.) in $CHCl_3$ (9 mL). The reaction flask was covered in aluminium foil and the reaction mixture was stirred at room temperature open to the atmosphere for 2.5 hours. The reaction mixture was filtered through a pad of celite, washed with $CHCl_3$ and the solvent was removed under reduced pressure to afford symmetrical carbonate **48** (197 mg, 57 % yield) as a yellow oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.40-7.22 (20H, m, ArC*H*), 4.19 (4H, t, *J* = 6.0 Hz, NCH_2CH_2O), 3.67 (8H, s, $PhCH_2NCH_2Ph$) and 2.78 (4H, t, *J* = 6.0 Hz, NCH_2CH_2O). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 155.1- (*C*=O), 139.2- (Ar*C*(1)), 128.7+ (Ar*C*H), 128.2+ (Ar*C*H), 127.0+ (Ar*C*H), 65.9- (NCH_2CH_2O), 58.7- ($PhCH_2NCH_2Ph$) and 51.6- (NCH_2CH_2O). **HRMS** (+ESI) Found MH^+ 531.2704, $C_{33}H_{36}N_2NaO_3$ requires *MH* 531.2624 (15 ppm). **Rf** (20 % EtOAc in light petroleum (b.p. 40-60 ^oC)) 0.5.

Synthesis of *N,N*-dibenzyl-2-chloroethanamine 49



41 **49**

Method A

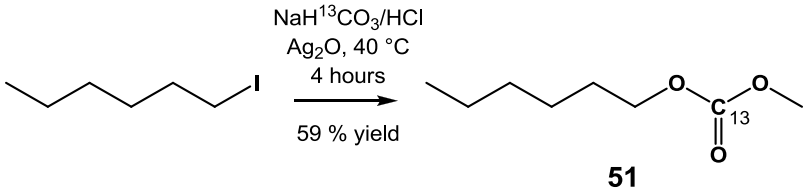
Iodide intermediate **41** (135 mg, 0.39 mmol, 1 eq.) was dissolved in $CHCl_3$ (4 mL) and CO_2 was bubbled through. MeOH (0.39 mL, 9.75 mmol, 25 eq.) was added followed by AgCl (62 mg, 0.43 mmol, 1.1 eq.) and the reaction mixture was stirred under a CO_2 atmosphere at room temperature for 16 hours. The resulting cloudy pale yellow mixture was filtered through a pad of celite and the solvent was removed under reduced pressure to collect chloride product **49** (87 mg, 87 % yield) as a clear yellow oil.

Method B

Iodide intermediate **41** (82 mg, 0.23 mmol, 1 eq.) was dissolved in MeCN (2 mL) and CO_2 was bubbled through. MeOH (0.23 mL, 5.75 mmol, 25 eq.) was added followed by AgCl (36 mg, 0.25 mmol, 1.1 eq.) and the reaction mixture was stirred under a CO_2 atmosphere at room temperature for 16 hours. The reaction mixture was filtered through a pad of celite, washed with EtOAc and the solvent was removed under reduced pressure to collect chloride product **49** (60 mg, 99 % yield) as a clear yellow oil.

^{1}H NMR (400 MHz; $CDCl_3$): δ_H 7.37 (4H, d, *J* = 7.0 Hz, ArC(2)*H*), 7.31 (4H, t, *J* = 8.0 Hz, ArC(3)*H*), 7.24 (2H, t, *J* = 7.0 Hz, ArC(4)*H*), 3.65 (4H, s, PhCH_2NCH_2Ph), 3.48 (2H, t, *J* = 7.0 Hz, NCH_2CH_2Cl) and 2.83 (2H, t, *J* = 7.0 Hz, NCH_2CH_2Cl). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 139.4- (Ar*C*(1)), 129.0+ (Ar*C*(4)H), 128.6+ (Ar*C*(3)H), 127.4+ (Ar*C*(2)H), 59.0- (PhCH_2NCH_2Ph), 55.6- (NCH_2CH_2Cl) and 42.1- (NCH_2CH_2Cl). **MS** *m/z* (+ESI) 260 (100 %, MH^+ (^{35}Cl)), 262 (31 %, MH^+ (^{37}Cl)) and 224 (12 %, aziridinium$^+$). **HRMS** (+ESI) Found MH^+ 260.1185, $C_{16}H_{19}{}^{35}ClN$ requires *MH* 260.1206. **IR** ν_{max}(liquid film): 3003 (CH) and 1601 (C=C). **Rf** (20 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.6. Spectroscopic data are consistent with those reported by Bernier *et al.*[163]

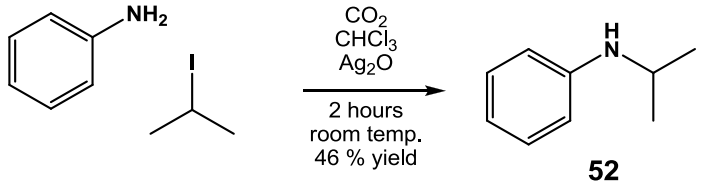
Synthesis of hexyl methyl [^{13}C]-carbonate 51



51

To a solution of 1-iodohexane (212 mg, 1 mmol, 1 eq.) and Ag_2O (278 mg, 1.2 mmol, 1.2 eq.) in MeOH (2.02 mL) was bubbled CO_2 generated from $NaH^{13}CO_3$ (425 mg, 5 mmol, 5 eq.) and 3M $HCl_{(aq.)}$. The suspension was stirred at 40 ^{o}C for 4 hours. The reaction mixture was filtered through filter paper and cotton wool, washed with MeOH and the solvent was removed under reduced pressure to afford carbonate **51** (96 mg, 60 % yield) as a colourless oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 4.12 (2H, t, *J* = 7.0 Hz, C*H*$_2$O), 3.76 (3H, s, OCH_3), 1.67 - 1.63 (2H, m, CH_2), 1.30 - 1.27 (6H, m, CH_2) and 0.89 - 0.86 (3H, m, CH_3). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 155.8- (*C*=O), 68.2- (O*C*H$_2$), 54.5+ (O*C*H$_3$), 31.3- (*C*H$_2$), 28.6- (*C*H$_2$), 25.3- (*C*H$_2$), 22.4- (*C*H$_2$) and 13.9+ (*C*H$_3$). **HRMS** (+ESI) Found MNa$^+$ 184.1008, $^{12}C_7{}^{13}CH_{16}NaO_3$ requires *MNa* 184.0997.

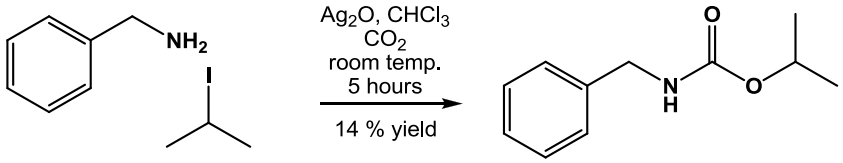
Synthesis of *N*-isopropylaniline 52



52

Ag_2O (510 mg, 2.2 mmol, 1.1 eq.) was added to a solution of 2-iodopropane (0.22 mL, 2.2 mmol, 1.1 eq.) and aniline (0.91 mL, 10 mmol, 5 eq.) in $CHCl_3$ (10 mL) and stirred at room temperature with an empty balloon on top. In a second sealed flask, 6M $HCl_{(aq.)}$ (1 mL) was added to $NaHCO_3$ (168 mg, 2 mmol, 1 eq.) and, using a double headed needle, the CO_2 produced was pushed into the main reaction flask and bubbled through the reaction mixture. The reaction mixture was left to stir at room temperature for 2 hours under an atmosphere of CO_2. Na_2SO_4 was added, the reaction mixture was filtered through a plug of celite and the celite was washed with $CHCl_3$. The solvent was removed under reduced pressure and, after column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], the compound **52** (124 mg, 46 % yield) was isolated as a yellow oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.17 (2H, t, *J* = 8.0 Hz, ArC(3)*H*), 6.68 (1H, d, *J* = 8.0 Hz, ArC(4)*H*), 6.59 (2H, d, *J* = 8.0 Hz, ArC(2)*H*), 3.61 (1H, sept, *J* = 6.0 Hz, C*H*) and 1.21 (6H, d, *J* = 6.0 Hz, CH_3). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 147.3- (Ar*C*(1)), 129.3+ (Ar*C*(3)H), 117.1+ (Ar*C*(4)H), 113.3+ (Ar*C*(2)H), 44.3+ (*C*H) and 23.0+ (*C*H_3). **Rf** (40 % EtOAc in light petroleum (b.p. 40-60 ^oC)) 0.6. Spectroscopic data are consistent with those reported by Williams *et al*.[164]

Synthesis of isopropyl N-benzylcarbamate 53

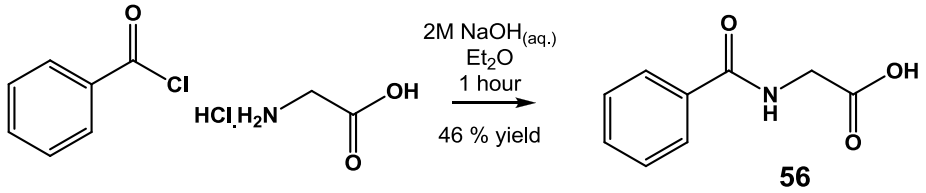


53

Ag_2O (510 mg, 2.2 mmol, 1.1 eq.) was added to a solution of 2-iodopropane (0.22 mL, 2.2 mmol, 1.1 eq.) and benzylamine (1.09 mL, 10 mmol, 5 eq.) in $CHCl_3$ (10 mL) and was stirred at room temperature with an empty balloon on top. In a second sealed flask, 6M $HCl_{(aq.)}$ (1 mL) was added to $NaHCO_3$ (168 mg, 2 mmol, 1 eq.) and, using a double headed needle, the CO_2 produced was pushed into the main reaction flask. The reaction mixture was left to stir at room temperature for 5 hours. Na_2SO_4 was added, the reaction mixture filtered through plug of celite and the celite was washed with $CHCl_3$. The solvent was removed under reduced pressure and, after column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], carbonate **53** (50 mg, 14 % yield) was isolated as a yellow oil (Klopotek *et al.* report this to be a solid with **m.p.** 34-35 ^oC[165]). **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.35-7.25 (5H, m, ArC*H*), 4.95 (1H, sept, *J* = 6.5 Hz, OC*H*), 4.93 (1H, m, N*H*), 4.36 (2H, d, *J* = 5.5 Hz, CH_2) and 1.24 (6H, d, *J* = 6.5 Hz, CH_3). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 156.3- (*C*=O), 138.7- (Ar*C*(1)), 128.6+ (Ar*C*H), 127.5+ (Ar*C*H), 127.4+ (Ar*C*H), 68.2+ (O*C*H), 44.9- (*C*H_2) and 22.1+ (*C*H_3). **MS** *m/z* (+ESI) 194 (17 %, MH^+), 216 (100 %, MNa^+). **HRMS** (+ESI) Found MH^+ 194.1169, $C_{11}H_{16}NO_2$ requires MH 194.1181 and found 216.0982, $C_{11}H_{15}NNaO_2$ requires *MNa* 216.1000. **IR** ν_{max}(liquid film): 3447 (NH), 3055 (CH), 1721 (C=O) and 1605 (C=C). **Rf** (60 % EtOAc in light petroleum (b.p. 40-60 ^oC)) 0.7. Spectroscopic data are consistent with those reported by Hyde *et al.*[166]

7.3.2. Acylation - Schotten-Baumann Reactions

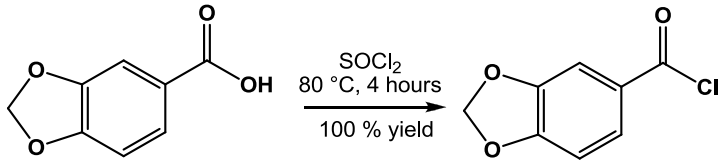
Synthesis of 2-benzamidoacetic acid 56



56

Following a procedure reported by Albrecht *et al.*,[78] glycine.HCl (223 mg, 2 mmol, 1 eq.) dissolved in 2M $NaOH_{(aq.)}$ (2 mL) was added dropwise to benzoyl chloride (0.23 mL, 2 mmol, 1 eq.) dissolved in Et_2O (1 mL). The reaction mixture was left to stir at room temperature for 1 hour. 6M $HCl_{(aq.)}$ was added and the solid filtered and washed with CH_2Cl_2 to collect **56** (165 mg, 46 % yield) as a white solid, **m.p.** 187-189 ^oC [lit.[167] 188 ^oC]. **^{1}H NMR** (400 MHz, CD_3OD): δ_H 7.86-7.83 (2H, m, ArC(2)*H*), 7.55 (1H, tt, *J* = 7.5, 1.5 Hz, ArC(4)*H*), 7.48-7.44 (2H, m, ArC(3)*H*) and 4.11 (2H, s, CH_2). **^{13}C NMR** (100 MHz, CD_3OD): δ_C 172.0- ((*C*O)OH), 170.6- ((*C*=O)N), 135.1- (Ar*C*(1)H), 133.0+ (Ar*C*(4)H) 129.6+ (Ar*C*(3)H), 128.4+ (Ar*C*(2)H) and 42.4- (*C*H_2). **MS** *m/z* (ESI+) 202 (76 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 202.0498, $C_9H_9NNaO_3$ requires *MNa* 202.0480.

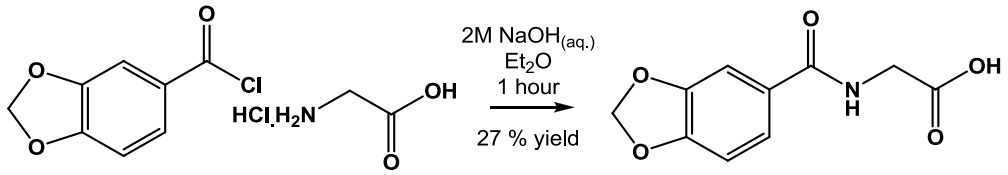
Synthesis of 3,4-methylenedioxybenzoyl chloride 57



57

Piperonylic acid (332 mg, 2 mmol, 1 eq.) in $SOCl_2$ (2 mL) with 1 drop of DMF were heated at 80 ^oC under an atmosphere of N_2 for 4 hours. The reaction mixture was left to cool and the solvent was removed under reduced pressure to afford acid chloride **57** (369 mg, 100 % yield) as a white solid, m.p. 77-79 ^oC [lit.[168] 78-79 ^oC]. **^{1}H NMR** (400 MHz, $CDCl_3$): δ_H 7.78 (1H, dd, *J* = 8.5 and 2.0 Hz, ArC(6)*H*), 7.50 (1H, d, *J* = 2.0 Hz, ArC(2)*H*), 6.88 (1H, d, *J* = 8.5 Hz, ArC(5)*H*) and 6.10 (2H, s, OCH_2O). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 166.8- (*C*=O), 153.9- (*C*), 148.4- (*C*), 128.9+ (Ar*C*(6)H), 127.2- (*C*), 110.6+ (Ar*C*(2)H), 108.3+ (Ar*C*(5)H) and 102.6- (O*C*H_2O).

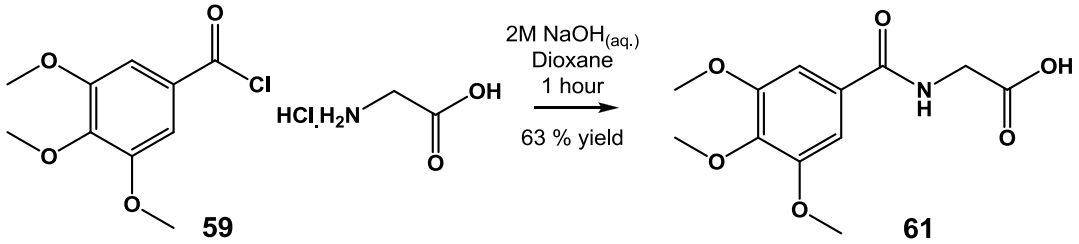
Synthesis of 2-(benzo[*d*][1,3]dioxole-5-carboxamido)acetic acid 58



57 **58**

Following a procedure reported by Albrecht *et al.*[78] glycine.HCl (223 mg, 2 mmol, 1 eq.) dissolved in 2M $NaOH_{(aq.)}$ (2 mL) was added dropwise to acid chloride **57** (369 mg, 2 mmol, 1 eq.) dissolved in Et_2O (1 mL). The reaction mixture was left to stir at room temperature for 1 hour. 6M $HCl_{(aq.)}$ was added and the solid was filtered and washed with CH_2Cl_2 to collect **58** (119 mg, 27 % yield) as a white solid m.p. 174-177 ^{o}C [lit.[169] 178 ^{o}C]. **^{1}H NMR** (400 MHz, CD_3OD): δ_H 7.45-7.42 (1H, m, ArC(6)*H*), 7.32 (1H, s, ArC(2)*H*), 6.89-6.86 (1H, m, ArC(5)*H*) 6.03-6.02 (2H, m, OCH_2O) and 4.05-4.04 (2H, m, C*H*$_2$). **^{13}C NMR** (100 MHz, CD_3OD): δ_C 172.1- ((*C*=O)OH), 169.8- ((*C*=O)N), 152.3- (*C*), 149.5- (*C*), 129.0- (*C*), 123.6+ (Ar*C*(6)H), 109.0+ (Ar*C*(5)H), 108.5+ (Ar*C*(2)H), 103.3- (O*C*H_2O) and 42.5- (*C*H_2).

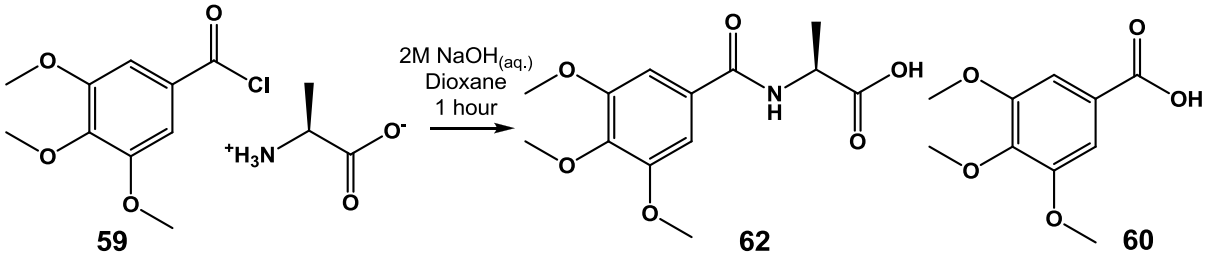
Synthesis of 2-(3,4,5-trimethoxybenzamido)acetic acid 61



59 61

Glycine.HCl (1.12 g, 10 mmol, 1 eq.) dissolved in 2M $NaOH_{(aq.)}$ (10 mL) was added dropwise to 3,4,5-trimethoxybenzoyl chloride **59** (2.31 g, 10 mmol, 1 eq.) dissolved in 1,4-dioxane (5 mL). The reaction mixture was left to stir at room temperature for 1 hour. 6M $HCl_{(aq.)}$ was added and the solid was filtered and washed with CH_2Cl_2 to collect **61** (1.70 g, 63 % yield) as a white solid, **m.p.** 221-223 ^{o}C.[170] **^{1}H NMR** (400 MHz, $(CD_3)_2SO$): δ_H 8.79 (1H, t, *J* = 5.5 Hz, N*H*), 7.20 (2H, s, ArC(2)*H*), 3.90 (2H, d, *J* = 6.0 Hz, CH_2), 3.81 (6H, s, OCH_3) and 3.69 (3H, s, OCH_3). **^{13}C NMR** (100 MHz, $(CD_3)_2SO$): δ_C 171.3- ((*C*=O)OH), 165.8- ((*C*=O)N), 152.6- (*C*),140.2- (*C*), 129.0- (*C*), 104.9+ (Ar*C*(2)H), 60.1+ (OCH_3), 56.0+ (OCH_3) and 41.2- (*C*H_2). **MS** *m/z* (ESI+) 292 (32 %, MNa^+). **HRMS** (+ESI) Found MH^+ 270.0969, $C_{12}H_{16}NO_6$ requires *MH* 270.0978 and found MNa^+ 292.0783, $C_{12}H_{15}NNaO_6$ requires *MNa* 292.0797.

Synthesis of *(S)*-2-(3,4,5-trimethoxybenzamido)propanoic acid 62



59 **62** **60**

L-Alanine (356 mg, 4 mmol, 2 eq.) dissolved in 2M $NaOH_{(aq.)}$ (3 mL, 6 mmol, 3 eq.) was added dropwise to 3,4,5-trimethoxybenzoyl chloride **59** (461 mg, 2 mmol, 1 eq.) dissolved in 1,4-dioxane (1 mL). The reaction mixture was left to stir at room temperature for 1 hour. 6M $HCl_{(aq.)}$ was added and the solid filtered to collect 468 mg of a white solid.

Ratio by crude NMR: **85 % product 62 (73 % yield)**
15 % 3,4,5-trimethoxybenzoic acid 60 (13 % yield)

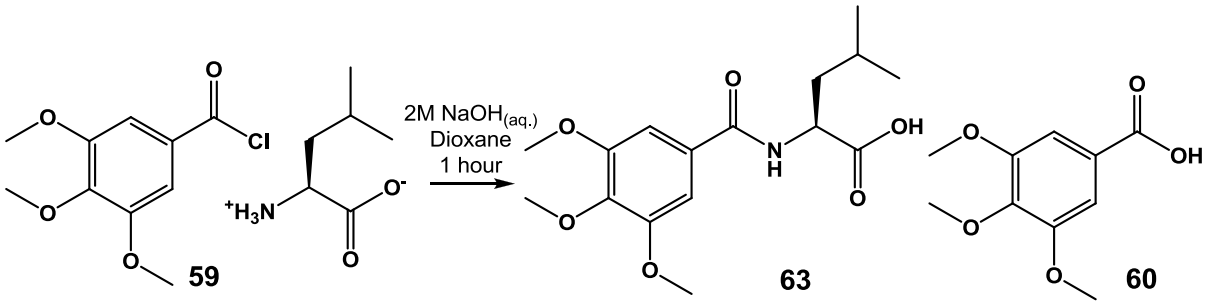
3,4,5-Trimethoxybenzoic acid 60

^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ_H 12.55 (1H, br. s. O*H*), 7.23 (2H, s, ArC(2)*H*), 3.82 (6H, s, OCH_3) and 3.73 (3H, s, OCH_3). **^{13}C NMR** (100 MHz, $(CD_3)_2SO$): δ_C 166.9- (*C*=O), 152.6- (*C*), 141.5- (*C*), 125.9- (*C*), 106.6+ (Ar*C*(2)H), 66.3+ (O*C*H_3) and 55.9+ (O*C*H_3).

Product 62[171]

^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ_H 12.55 (1H, br. s., O*H*), 8.57 (1H, d, *J* = 7.0 Hz, N*H*), 7.23 (2H, s, ArC(2)*H*), 4.43 (1H, quint, *J* = 7.5 Hz, C*H*), 3.84 (6H, s, OCH_3), 3.71 (3H, s, OCH_3) and 1.40 (3H, d, *J* = 7.5 Hz, CH_3). **^{13}C NMR** (100 MHz, $(CD_3)_2SO$): δ_C 174.2- ((*C*=O)OH), 165.4- ((*C*=O)N), 152.5- (*C*),140.2- (*C*), 129.0- (*C*), 105.1+ (Ar*C*(2)H), 60.1+ (O*C*H_3), 56.0+ (O*C*H_3), 48.2+ (*C*H) and 17.0+ (*C*H_3). **MS** *m/z* (+ESI) 306 (100 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 306.0936, $C_{13}H_{17}NNaO_6$ requires *MNa* 306.0954.

Synthesis of *(S)*-4-methyl-2-(3,4,5-trimethoxybenzamido)pentanoic acid 63



L-Leucine (262 mg, 2 mmol, 2 eq.) dissolved in 2M $NaOH_{(aq.)}$ (1.5 mL, 3 mmol, 3 eq.) was added dropwise to 3,4,5-trimethoxybenzoyl chloride **59** (231 mg, 1 mmol, 1 eq.) dissolved in 1,4-dioxane (1 mL). The reaction mixture was left to stir at room temperature for 1 hour. 6M $HCl_{(aq.)}$ was added and the solid filtered to collect 287 mg of a yellow solid.

Ratio by crude NMR: **66 % product 63 (66 % yield)**

34 % 3,4,5-trimethoxybenzoic acid 60 (34 % yield)

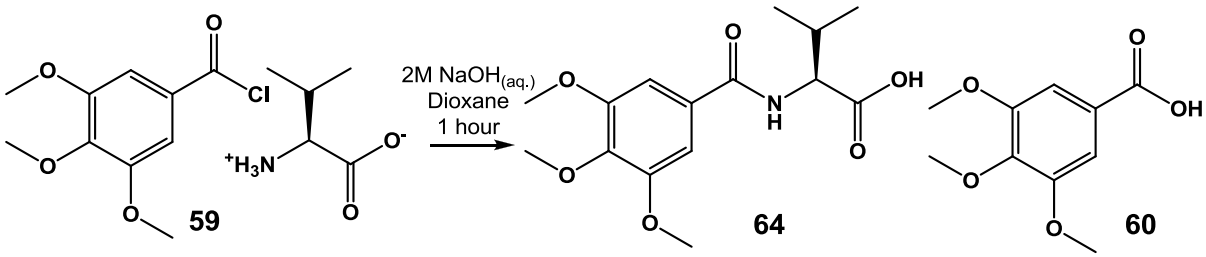
3,4,5-Trimethoxybenzoic acid 60

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 10.62 (1H, br. s. O*H*), 7.31 (2H, s, ArC(2)*H*), 3.89 (3H, s, OCH_3) and 3.83 (6H, s, OCH_3). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 170.8- (*C*=O), 152.9- (*C*), 142.9- (*C*), 124.1- (*C*), 107.4+ (Ar*C*(2)H), 60.8+ (O*C*H_3) and 56.2+ (O*C*H_3).

Product 63[172]

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 10.62 (1H, br. s., O*H*), 7.03 (2H, s, ArC(2)*H*), 6.89 (1H, d, *J* = 8.0 Hz, N*H*), 4.84-4.79 (1H, m, (NH)C*H*), 3.87 (3H, s, OCH_3), 3.84 (6H, s, OCH_3), 1.80-1.66 (3H, m, C*H* and CH_2), 0.95 (3H, d, *J* = 6.0 Hz, CH_3) and 0.95 (3H, d, *J* = 6.0 Hz, CH_3). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 177.0- ((*C*=O)OH), 167.5- ((*C*=O)N), 152.9- (*C*), 141.4- (*C*), 128.7- (*C*), 104.8+ (Ar*C*(2)H), 60.8+ (O*C*H_3), 56.3+ (O*C*H_3), 51.4+ ((NH)C*H*), 41.2- (*C*H_2), 25.0+ (*C*H), 22.7+ (*C*H_3) and 21.8+ (*C*H_3). **MS** *m/z* (+ESI) 348 (44 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 348.1398, $C_{16}H_{23}NNaO_6$ requires *MNa* 348.1423 (7 ppm).

Synthesis of *(S)*-3-methyl-2-(3,4,5-trimethoxybenzamido)butanoic acid 64



L-Valine (234 mg, 2 mmol, 2 eq.) dissolved in 2M $NaOH_{(aq.)}$ (1.5 mL, 3 mmol, 3 eq.) was added dropwise to 3,4,5-trimethoxybenzoyl chloride **59** (231 mg, 1 mmol, 1 eq.) dissolved in 1,4-dioxane (1 mL). The reaction mixture was left to stir at room temperature for 1 hour. 6M $HCl_{(aq.)}$ was added and the solid filtered to collect 218 mg of a white solid.

Ratio by crude NMR: **72 % product 64 (55 % yield)**
28 % 3,4,5-trimethoxybenzoic acid 60 (22 % yield)

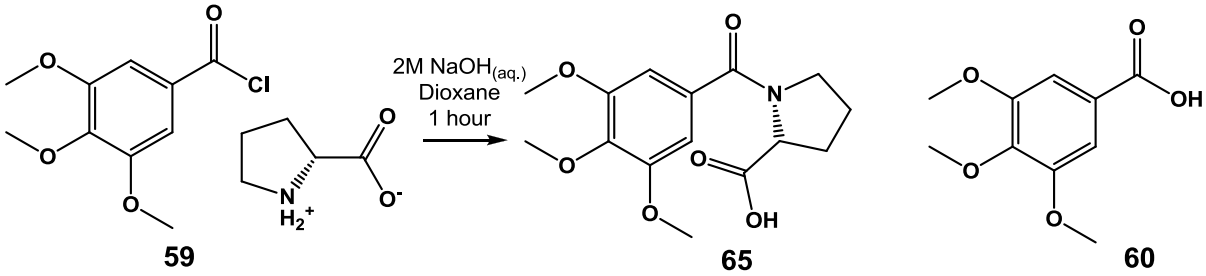
3,4,5-Trimethoxybenzoic acid 60

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 10.43 (1H, br. s. O*H*), 7.31 (2H, s, ArC(2)*H*), 3.89 (3H, s, OCH_3) and 3.84 (6H, s, OCH_3). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 170.6- (*C*=O), 152.9- (*C*), 142.9- (*C*), 124.1- (*C*), 107.4+ (Ar*C*(2)H), 60.8+ (O*C*H_3) and 56.2+ (O*C*H_3).

Product 64[173]

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 10.43 (1H, br. s., O*H*), 7.02 (2H, s, ArC(2)*H*), 6.77 (1H, d, *J* = 8.5 Hz, N*H*), 4.84-4.79 (1H, m, (NH)C*H*), 3.87 (3H, s, OCH_3), 3.84 (6H, s, OCH_3), 1.80-1.66 (1H, m, C*H*), 1.07 (3H, d, *J* = 7.0 Hz, CH_3) and 1.00 (3H, d, *J* = 7.0 Hz, CH_3). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 175.4- ((*C*=O)OH), 167.7- ((*C*=O)N), 153.2- (*C*),141.4- (*C*), 129.1- (*C*), 104.8+ (Ar*C*(2)H), 60.8+ (O*C*H_3), 56.3+ (O*C*H_3), 57.7+ ((NH)C*H*), 31.2+ (*C*H), 19.0+(*C*H_3) and 17.9+ (*C*H_3). **MS** *m/z* (+ESI) 312 (44 %, MH^+) and 334 (100 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 334.1250, $C_{15}H_{21}NNaO_2$ requires *MNa* 334.1266.

Synthesis of N-(3,4,5-trimethoxybenzoyl)-D-proline 65



59 **65** **60**

(*R*)-Pyrrolidine-2-carboxylic acid (460 mg, 4 mmol, 2 eq.) dissolved in 2M $NaOH_{(aq.)}$ (3 mL, 6 mmol, 3 eq.) was added dropwise to 3,4,5-trimethoxybenzoyl chloride **59** (461 mg, 2 mmol, 1 eq.) dissolved in 1,4-dioxane (2 mL). The reaction mixture was left to stir at room temperature for 1 hour. 6M $HCl_{(aq.)}$ was added and the solid filtered to collect 554 mg of a white solid.

Ratio by crude NMR: **67 % product 65 (67 % yield)**
33 % 3,4,5-trimethoxybenzoic acid 60 (33 % yield)

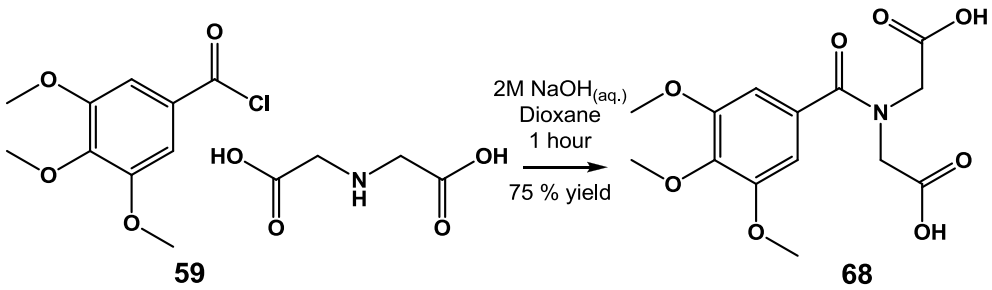
3,4,5-Trimethoxybenzoic acid 60

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 10.40 (1H, br. s., O*H*), 7.31 (2H, s, ArC(2)*H*), 3.89 (3H, s, OCH_3) and 3.84 (6H, s, OCH_3). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 170.6- (*C*=O), 152.9- (*C*), 142.9- (*C*), 124.1- (*C*), 107.4+ (Ar*C*(2)H), 60.8+ (O*C*H_3) and 56.2+ (O*C*H_3).

Product 65

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 6.78 (2H, s, ArC*H*), 4.70 (1H, t, *J* = 7.0 Hz, NC*H*), 3.86 (6H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.61 (2H, t, *J* = 6.5 Hz, NCH_2), 2.29-2.24 (2H, m, CH_2) and 2.08-1.86 (2H, m, CH_2). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 174.1- ((*C*=O)OH), 170.7- ((*C*=O)N), 153.1- (*C*), 140.0- (*C*), 130.4- (*C*), 104.9+ (Ar*C*(2)H), 60.8+ (O*C*H_3), 59.8+ (N*C*H), 56.3+ (O*C*H_3), 56.2+ (O*C*H_3), 50.5- (N*C*H_2), 28.5- (*C*H_2) and 25.2- (*C*H_2). **MS** *m/z* (+ESI) 310 (15 %, MH^+) and 332 (99 %, MNa^+). **HRMS** (+ESI) Found MH^+ 310.1283, $C_{15}H_{20}NO_6$ requires MH 310.1291.

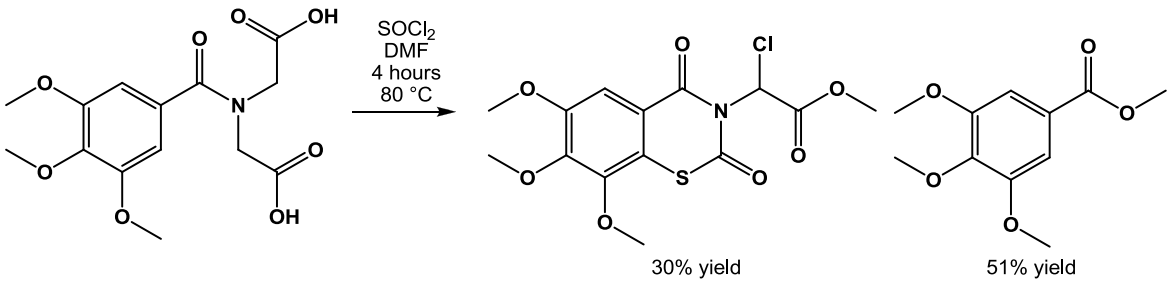
Synthesis of N-(3,4,5-trimethoxybenzoyl)iminodiacetic acid 68



59 68

Iminodiacetic acid (266 mg, 2 mmol, 2 eq.) in 2M $NaOH_{(aq.)}$ (3 mL) was added dropwise to 3,4,5-trimethoxybenzoyl chloride **59** (231 mg, 1 mmol, 1 eq.) in 1,4-dioxane (1 mL). The reaction mixture was stirred for 1 hour at room temperature and acidified using 6M $HCl_{(aq.)}$. The reaction mixture was filtered and the white solid was washed with CH_2Cl_2 (10 mL) to collect **68** (245 mg, 75 % yield) as a white solid, **m.p.** 156-159 ^{o}C. **^{1}H NMR** (400 MHz, $(CD_3)_2SO$): δ_H 12.92 (2H, br.s. O*H*), 6.62 (2H, s, ArC(2)*H*), 4.10 (2H, s, CH_2), 4.02 (2H, s, CH_2), 3.76 (6H, s, OCH_3) and 3.70 (3H, s, OCH_3). **^{13}C NMR** (100 MHz, $(CD_3)_2SO$): δ_C 171.2- (*C*=O), 170.7- (*C*=O), 170.1- (*C*=O), 152.8- (*C*), 138.8- (*C*), 130.3- (*C*), 104.1+ (Ar*C*(2)H), 60.1+ (O*C*H_3), 55.9+ (O*C*H_3), 51.7- (*C*H_2) and 48.0- (CH_2). **MS** *m/z* (ESI+) 350 (26 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 350.0890, $C_{14}H_{17}NNaO_8$ requires *MNa* 350.0852 (11 ppm). **IR** ν_{max}(liquid film): 2940 (OH), 1715 (C=O), 1689 (C=O) and 1589 (C=C).

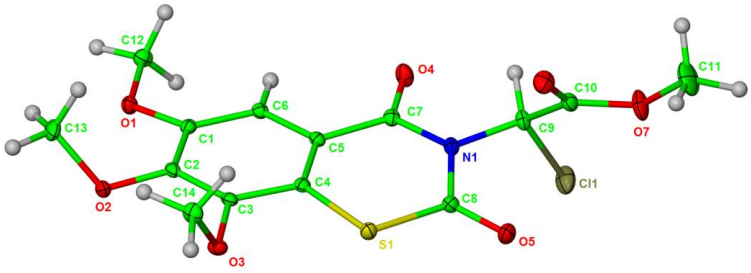
Synthesis of methyl 2-chloro-2-(6,7,8-trimethoxy-2,4-dioxo-2H-benzo[*e*][1,3]thiazin-3(*4H*)-yl)acetate 69 and methyl 3,4,5-trimethoxybenzoate 70



68 **69** **70**

Di-acid **68** (327 mg, 1 mmol, 1 eq.) in $SOCl_2$ (1 mL) and a drop of DMF were heated at 80 ^oC under an atmosphere of N_2 for 4 hours. The reaction mixture was allowed to cool, the reaction mixture was quenched with MeOH and the solvent was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], the cyclic product **69** (112 mg, 30 % yield) as a clear orange oil and ester **70** (115 mg, 51 % yield) as a brown solid were isolated.

Cyclic product 69: ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.67 (1H, s, ArC*H*), 7.18 (1H, s, ClC*H*), 3.99 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 3.94 (3H, s, OCH_3) and 3.84 (3H, s, (CO)OC*H*$_3$). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 164.9- ((*C*=O)OCH_3), 164.4- (S(*C*=O)N), 161.3- (N(*C*=O)), 153.1- (*C*), 147.6- (*C*), 146.8- (*C*), 119.5- (*C*), 116.5- (*C*), 109.3+ (ArCH), 61.3+ (O*C*H$_3$), 61.2+ (OCH_3), 60.7+ (Cl*C*H), 56.4+ (OCH_3) and 54.1+ (OCH_3). **MS** *m/z* (+ESI) 398 (100 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 398.0048, $C_{14}H_{14}ClNNaO_7S$ requires *MNa* 398.0077 (7 ppm). **Crystal Structure:**



Ester 70: m.p. 79-83 ^{o}C [lit.[174] 81-83 ^{o}C]. **^{1}H NMR** (400 MHz, $CDCl_3$): δ_H 7.28 (2H, s, ArC*H*) and 3.88 (12H, s, OCH_3). Spectroscopic data are consistent with those reported by Keck *et al.*[175]

7.4. Chapter Three - A/B Analogues of Dihydroisoquinolinones

7.4.1. Indanone Synthesis

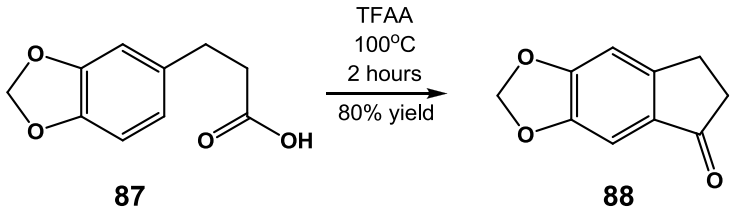
General Procedure 4, Intramolecular Indanone Synthesis

The required acid (1 eq.) in TFAA (1 eq.) was heated at 100 ^{o}C for 2 hours in a sealed pressure tube. On cooling, the reaction mixture was transferred to a flask using $CHCl_3$ and the solvent was removed under reduced pressure. After column chromatography, the desired indanone was isolated.

General Procedure 5, Indanone Synthesis

The required acid (1 eq.) in (TFAA/ TFA) was heated at 100 ^{o}C in a sealed pressure tube. $CHCl_3$ or MeOH was added and the solvent was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column] the desired indanone was isolated.

Synthesis of 6,7-dihydro-5H-indeno[5,6-*d*][1,3]dioxol-5-one 88



87 **88**

Method A

Following a procedure reported by Dallemagne *et al.*[90] 3-(1,3-benzodioxol-5-yl)propanoic acid **87** (194 mg, 1 mmol, 1 eq.) in TFAA: TFA (0.5 mL: 0.5 mL) was heated at 50 ^{o}C for 2.5 hours. On cooling, the reaction mixture was adjusted to pH 12 using 2M $NaOH_{(aq.)}$ and the aqueous layer extracted with $CHCl_3$ (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried on Na_2SO_4, filtered and the solvent was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the desired indanone **88** (36 mg, 20 % yield) was isolated as a yellow solid.

Method B

3-(1,3-Benzodioxol-5-yl)propanoic acid **87** (194 mg, 1 mmol, 1 eq.) in TFAA: TFA (0.5 mL: 0.5 mL) was heated at 50 ^{o}C for 3 hours. Methanol was added to quench the reaction mixture and the solvent was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the desired indanone **88** (141 mg, 81 % yield) was isolated as a yellow solid.

Method C

Following **General Procedure 5**, using 3-(1,3-benzodioxol-5-yl)propanoic acid **87** on a 1 mmol scale, in TFAA: TFA (0.5 mL: 1.0 mL) for 1 hour, using $CHCl_3$ to transfer the reaction mixture, the indanone **88** (148 mg, 84 % yield) was isolated as a yellow solid.

Method D

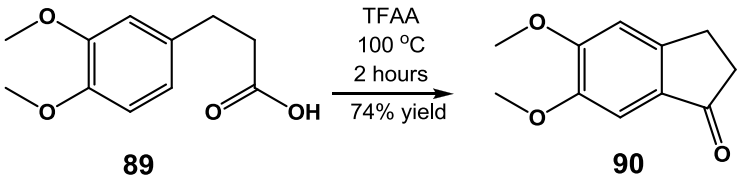
3-(1,3-Benzodioxol-5-yl)propanoic acid **87** (388 mg, 2 mmol, 1 eq.) in TFAA: TFA (1.0 mL: 2.0 mL) was heated at 100 ^{o}C in a sealed pressure tube for 2 hours. $CHCl_3$ was added and the solvent was removed under reduced pressure. After column chromatography (x 2) [silica, CH_2Cl_2 - MeOH gradient column], the desired indanone **88** (255 mg, 72 % yield) was isolated as a yellow solid.

Method E - Optimised Method

Following **General Procedure 4**, using 3-(1,3-benzodioxol-5-yl)propanoic acid **87** on a 1 mmol scale, after column chromatography [silica, CH_2Cl_2 - MeOH gradient column], the indanone **88** (141 mg, **80 % yield**) was isolated as a yellow solid.

m.p. 161-164 ^{o}C [lit.[176] 162-163 ^{o}C]. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.10 (1H, s, (C=O)CArC*H*), 6.83 (1H, s, CH_2CArC*H*), 6.06 (2H, s, OC*H_2*O), 3.02 (2H, t, *J* = 5.5 Hz, (CO)CH_2C*H_2*) and 2.64-2.62 (2H, m, (CO)C*H_2*CH_2). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 204.9- (*C*=O), 154.2- (Ar*C*OCH_2), 152.6- (Ar*C*CH_2 or Ar*C*(CO)), 148.2- (Ar*C*OCH_2), 131.7- (Ar*C*CH_2 or Ar*C*(CO)), 105.7+ (CH_2CAr*C*H), 102.3+ ((C=O)CAr*C*H), 102.2- (O*C*H_2O), 36.7- ((CO)*C*$H_2$$CH_2$) and 25.8- ((CO)$CH_2$*C*$H_2$). **MS** *m/z* (+ESI) 177 (82 %, MH^+) and 199 (100 %, MNa^+). **HRMS** (+ESI) Found MH^+ 177.0543, $C_{10}H_{19}O_3$ requires *MH* 177.0552 and found MNa^+ 199.0362, $C_{10}H_8NaO_3$ requires *MNa* 199.0371. **IR** ν_{max}(liquid film): 2987 (CH) and 1698 (C=O). **Rf** (50 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.5. Spectroscopic data are consistent with those reported by Odedra *et al.*[177]

Synthesis of 5, 6-dimethoxy-2,3-dihydro-1H-inden-1-one 90



89 **90**

Method A

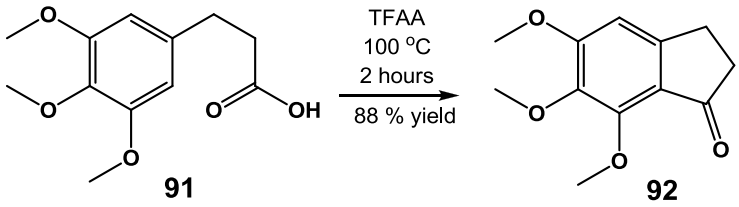
Following **General Procedure 5**, using 3-(3,4-dimethoxyphenyl)propanoic acid **89** on a 2 mmol scale, in TFAA: TFA (1.0 mL: 2.0 mL) for 16 hours, using $CHCl_3$ to transfer the reaction mixture, indanone **90** (252 mg, 66 % yield) was isolated as a yellow solid.

Method B – Optimised Method

Following **General Procedure 4**, using 3-(3,4-dimethoxyphenyl)propanoic acid **89** on a 2 mmol scale, after column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], indanone **90** (285 mg, **74 % yield**) was isolated as a yellow solid.

m.p. 115-118 ^oC [lit.[176] 115-117 ^oC]. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.15 (1H, s, (C=O)CArC*H*), 6.87 (1H, s, CH_2CArC*H*), 3.95 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 3.04 (2H, t, *J* = 5.5 Hz, (CO)CH_2CH_2) and 2.69-2.66 (2H, m, (CO)C$H_2$$CH_2$). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 202.7- (*C*=O), 155.7- (*C*OCH_3), 151.0- (*C*CH_2 or *C*(CO)), 149.4- (*C*OCH_3), 129.6- (*C*CH_2 or *C*(CO)), 107.4+ (CH_2CAr*C*H), 104.2+ ((C=O)CAr*C*H), 56.2+ (O*C*H_3), 56.0+ (O*C*H_3), 36.4- ((CO)*C*H_2CH_2) and 25.6- ((CO)CH_2*C*H_2). **MS** *m/z* (+ESI) 193 (100 %, MH^+). **HRMS** (+ESI) Found MH^+ 193.0851, $C_{11}H_{13}O_3$ requires *MH* 193.0810 (21 ppm). **IR** ν_{max}(liquid film): 2987 (CH), 1698 (C=O), 1606 (C=C) and 1030 (C-O). **Rf** (50 % EtOAc in light petroleum (b.p. 40-60 ^oC)) 0.3. Spectroscopic data are consistent with those reported by Fillion *et al.*[178]

Synthesis of 5,6,7-trimethoxy-2,3-dihydro-1H-inden-1-one 92



91 92

Method A

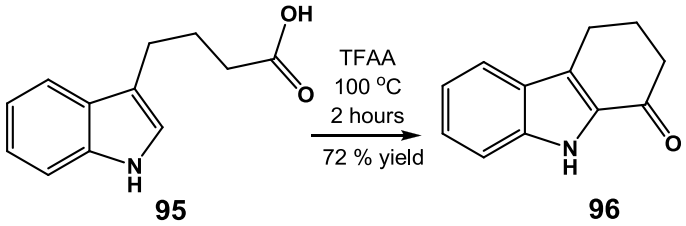
3-(3,4,5-Trimethoxyphenyl)propanoic acid **91** (240 mg, 1 mmol, 1 eq.) and TFAA (0.15 mL, 1.1 mmol, 1.1 eq.) in TFA (0.5 mL) were heated at 100 °C for 2 hours in a sealed pressure tube. On cooling, $CHCl_3$ was added and the solvent was removed under reduced pressure. H_2O (10 mL) and EtOAc (10 mL) were added and the reaction mixture was stirred at room temperature for 24 hours. The layer was extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried on Na_2SO_4 and filtered and the solvent was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 °C) - EtOAc gradient column], the indanone **92** (171 mg, 77 % yield) was isolated as a yellow solid.

Method B – Optimised Method

Following **General Procedure 4**, using 3-(3,4,5-trimethoxyphenyl)propanoic acid **91** on a 2 mmol scale, after column chromatography [silica, CH_2Cl_2 - MeOH gradient column], the indanone **92** (196 mg, **88 % yield**) was isolated as a yellow solid.

m.p. 111-114 °C [lit.[179] 111.5-113.5 °C]. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 6.65 (1H, s, ArC*H*), 4.00 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.81 (3H, s, OCH_3), 2.98 (2H, t, *J* = 6.4 Hz, (CO)CH_2CH_2) and 2.64-2.61 (2H, m, (CO)C$H_2$$CH_2$). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 203.5- (*C*=O), 159.8- (*C*OCH_3), 153.3- (*C*(CO)), 151.5- (*C*OCH_3), 140.5- (*C*OCH_3), 122.7- (*C*CH_2), 103.7+ (Ar*C*H), 61.8+ (O*C*H_3), 61.3+ (O*C*H_3), 56.2+ (O*C*H_3), 37.1- ((CO)*C*H_2CH_2) and 25.62- ((CO)CH_2*C*H_2). **MS** *m/z* (+ESI) 223 (100 %, MH^+) and 245 (41 %, MNa^+). **HRMS** (+ESI) Found MH^+ 223.0954, $C_{12}H_{15}O_4$ requires *MH* 223.0970 and found MNa^+ 245.0779, $C_{12}H_{14}NaO_4$ requires *MNa* 245.0790. **IR** ν_{max}(liquid film): 2987 (CH), 1694 (C=O), 1590 (C=C) and 1029 (C-O). **Rf** (50 % EtOAc in light petroleum (b.p. 40-60 °C)) 0.3. Spectroscopic data are consistent with those reported by Cui *et al.*[180]

Synthesis of 2,3,4,9-tetrahydro-1H-carbazol-1-one 96



95 **96**

Method A

4-(Indol-3-yl)butanoic acid **95** (1.22 g, 6 mmol, 1 eq.) in TFAA (0.92 mL, 6.6 mmol, 1.1 eq.) was heated at 100 ^{o}C in a sealed pressure tube for 4 hours. On cooling, $CHCl_3$ was added and the solvent was removed under reduced pressure. H_2O (20 mL) and EtOAc (20 mL) were added, the reaction mixture was adjusted to pH 7 using 2M $NaOH_{(aq.)}$ and stirred at room temperature for 16 hours. The layers were separated and the aqueous layer extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (20 mL), dried on Na_2SO_4 and filtered and the solvent was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column] followed by recrystallisation (EtOAc), compound **96** was isolated (592 mg, 53 % yield) as a yellow solid.

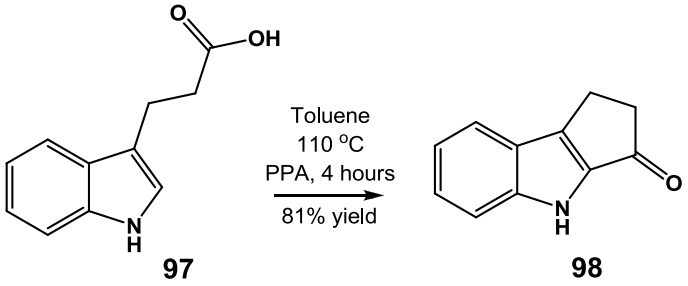
Method B - Optimised Method

Following **General Procedure 4**, using 4-(indol-3-yl)butanoic acid **95** on a 2 mmol scale, after column chromatography [silica, CH_2Cl_2 – MeOH gradient column], compound **96** (268 mg, **72 % yield**) was isolated as a pale yellow solid.

m.p. 170-172 ^{o}C [lit.[181] 168-170 ^{o}C]. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 9.31 (1H, br. s, N*H*), 7.66 (1H, d, *J* = 8.0 Hz, ArC(3)*H*), 7.45 (1H, dt, *J* = 8.0, 1.0 Hz, ArC(6)*H*), 7.37 (1H, t, *J* = 8.0 Hz, ArC(5)*H*), 7.15 (1H, t, *J* = 7.5 Hz, ArC(4)*H*), 3.01 (2H, t, *J* = 6.0 Hz, (CO)$CH_2CH_2CH_2$), 2.68 (2H, t, *J* = 6.0 Hz, (CO)$CH_2CH_2CH_2$) and 2.27 (2H, quint, *J* = 6.0 Hz, (CO)$CH_2CH_2CH_2$). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 191.5- (*C*=O), 137.9- (*C*), 131.2- (*C*), 129.6- (*C*), 127.0+ (Ar*C*(5)H), 125.8- (*C*), 121.3+ (Ar*C*(3)H), 120.3+ (Ar*C*(4)H), 112.6+ (Ar*C*(6)H), 38.2- ((CO)$CH_2CH_2CH_2$), 25.0- ((CO)$CH_2CH_2CH_2$) and 21.4- ((CO)$CH_2CH_2CH_2$). **MS** *m/z* (+ESI) 186 (43 %, MH^+) and 208 (100 %, MNa^+). **HRMS** (+ESI) Found MH^+ 186.0909, $C_{12}H_{12}NO$ requires *MH* 186.0919 and found MNa^+ 208.0726, $C_{12}H_{11}NNaO$ requires *MNa* 208.0738. **IR** ν_{max}(liquid film): 3450 (NH), 3054 (CH) and 1666

(C=O). **Rf** (50 % EtOAc in light petroleum (b.p. 40-60 ^{o}C) 0.5. Spectroscopic data are consistent with those reported by Li *et al.*[182]

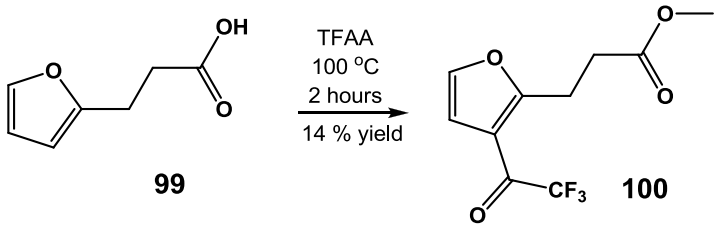
Synthesis of 1,2-dihydrocyclopenta[b]indol-3(*4H*)-one 98



97 **98**

Following a procedure reported by Maertens *et al.*[92] 3-(indole-3-yl)propanoic acid **97** (378 mg, 2 mmol, 1 eq.) was added to PPA (3.5 g, 35.7 mmol, 17.9 eq.) in toluene (20 mL) and the mixture was stirred at 110 ^{o}C for 4 hours. On cooling, ice water (80 mL) was added and the purple aqueous layer was extracted using CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with brine (30 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure to collect the desired compound **98** (278 mg, 81 % yield) as a beige solid, **m.p.** 255-258^{o}C [lit.[183] 250-252 ^{o}C]. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 8.98 (1H, br. s. N*H*), 7.75 (1H, dd, *J* = 8.0, 1.0 Hz, ArC(3)*H*), 7.52 (1H, dt, *J* = 8.5, 1.0 Hz, ArC(6)*H*), 7.44 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC(5)*H*), 7.23 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC(4)*H*), 3.17-3.14 (2H, m, (CO)$CH_2C\mathit{H_2}$) and 3.08-3.05 (2H, m, (CO)$C\mathit{H_2}CH_2$). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 194.5- (*C*=O), 127.4- (*C*), 147.2- (*C*), 143.8- (*C*), 127.4+ (Ar*C*(5)H), 123.6- (*C*), 121.6+ (Ar*C*(3)H), 120.8+ (Ar*C*(4)H), 113.5+ (Ar*C*(6)H), 41.0- ((CO)$\mathit{C}H_2CH_2$) and 20.1- ((CO)$CH_2\mathit{C}H_2$). **MS** *m/z* (+ESI) 172 (82 %, MH^+) and 194 (100 %, MNa^+). **HRMS** (+ESI) Found MH^+ 172.0755, $C_{11}H_{10}NO$ requires *MH* 172.0762 and found MNa^+ 194.0572, $C_{11}H_9NNaO$ requires *MNa* 194.0581. **IR** ν_{max}(liquid film): 3466 (NH), 3031 (CH) and 1682 (C=O). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C) 0.6. Spectroscopic data are consistent with those reported by Cui *et al.*[180]

Synthesis of 3-(4-trifluoroacetyl)furan-2-yl)propanoic acid 100



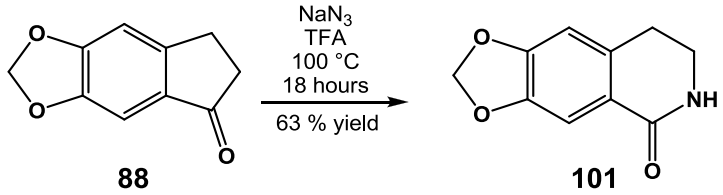
Following **General Procedure 4**, using 3-(furan-2-yl)propanoic acid **99** on a 2 mmol scale, using MeOH as the solvent to transfer the reaction mixture. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], compound **100** (72 mg, 14 % yield) was isolated as a brown oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.43 (1H, d, *J* = 4.0 Hz, OC*H*CH), 6.37 (1H, d, *J* = 4.0 Hz, OCHC*H*), 3.69 (3H, s, OCH_3), 3.09 (2H, t, *J* = 7.5 Hz, CH_2) and 2.74 (2H, t, *J* = 7.5 Hz, CH_2). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 172.1- (*C*=O), 172.1- (*C*=O), 164.0- (*C*), 145.9- (*C*), 126.0+ (O*C*HCH), 117.8- (*C*F_3), 110.1+ (OCH*C*H), 51.9+ (O*C*H_3), 31.4- (*C*H_2) and 23.8 (*C*H_2). **^{19}F NMR** (400MHz; $CDCl_3$): δ_F -73.3 (C*F*$_3$). **MS** *m/z* (+ESI) 251 (51 %, MH^+) and 273 (100 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 273.0343, $C_{10}H_9F_3NaO_4$ requires *MNa* 273.0351. **IR** ν_{max}(liquid film): 3054 (CH), 1739 (C=O), 1697 (C=O), 1509 (C=C) and 1265 (C-O). **Rf** (60 % EtOAc in light petroleum (b.p. 40-60 ^{o}C) 0.8.

7.4.2. Schmidt Reactions

General Procedure 6, Schmidt Reaction

The required indanone (1 eq.) and NaN_3 (2 eq.) in TFA (3 mL/ 1 mmol) were heated at 100 ^oC in a sealed pressure tube for 18 hours. The reaction mixture was cooled and sat. aq. $NaHCO_3$ was added dropwise followed by EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc (x 2). The combined organic layers were washed with brine, dried on Na_2SO_4, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc - MeOH gradient column], the desired lactam was isolated.

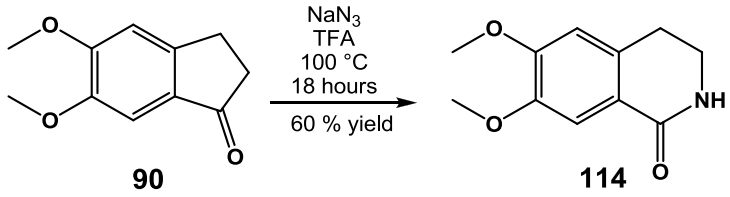
Synthesis of 7,8-dihydro-[1,3]dioxolo[4,5-*g*]isoquinolin-5(*6H*)-one 101



88 **101**

Following **General Procedure 6,** using indanone **88** (176 mg, 1 mmol, 1 eq.) and NaN_3 (130 mg, 2 mmol, 2 eq.) in TFA (3 mL) desired lactam **101** (120 mg, 63 % yield) was isolated as a yellow solid, **m.p.** 184-187 ^oC [lit.[93] 185-187 ^oC]. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.49 (1H, s, (ArC*H*C(CO)), 6.70 (1H, br.s. N*H*), 6.64 (1H, s, ArC*H*CCH_2), 5.98 (2H, s, OCH_2O), 3.52 (2H, br.s, NCH_2CH_2) and 2.89 (2H, t, *J* = 6.0 Hz, NCH_2CH_2). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 166.2- (*C*O), 150.8- (*C*), 146.8- (*C*), 134.6- (*C*), 122.8- (*C*), 107.9+ (Ar*C*HC(CO)), 107.2+ (Ar*C*HCCH_2), 101.4- (O*C*H_2O), 40.2- (N*C*H_2) and 28.4- (Ar*C*H_2). **MS** *m/z* (ESI+) 192 (100 %, MH^+) and 214 (50 %, MNa^+). **HRMS** (+ESI) Found MH^+ 192.0648, $C_{10}H_9NO_3$ requires *MH* 192.0661 (7 ppm). **IR** ν_{max}(liquid film): 3419 (NH), 2987 (CH) and 1666 (C=O). **Rf** (100 % EtOAc) 0.3. Spectroscopic data are consistent with those reported by Judd *et al*.[93]

Synthesis of 6,7-dimethoxy-3,4-dihydroisoquinolin-1(2H)-one 114



Method A - Optimised Method

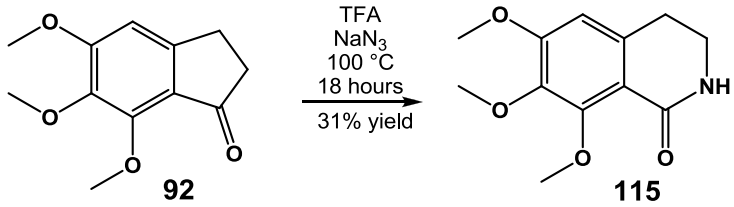
Following **General Procedure 6,** using indanone **90** (600 mg, 3.12 mmol, 1 eq.) and NaN_3 (406 mg, 6.24 mmol, 2 eq.) in TFA (9 mL) desired lactam **114** (387 mg, 60 % yield) was isolated as a yellow solid.

Method B

At 0 ^oC, NaN_3 (107 mg, 1.64 mmol, 2 eq.) was added to indanone **90** (158 mg, 0.82 mmol, 1 eq.) in H_2SO_4 (2 mL) and the reaction mixture was stirred at room temperature for 16 hours. The reaction mixture was diluted with water (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure to collect 142 mg of crude. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], the desired lactam **114** (111 mg, 65 % yield) was isolated as a yellow solid.

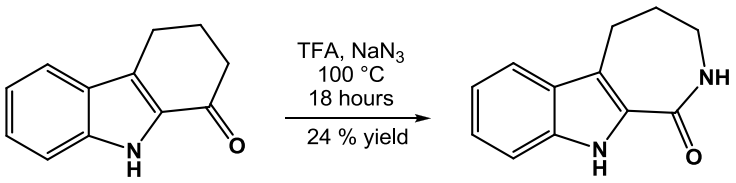
m.p. 175-177 ^oC [lit.[93] 174-177 ^oC]. **^{1}H NMR** (400 MHz, $CDCl_3$): δ_H 7.57 (1H, s, ArC*H*C(CO)), 6.67 (1H, s, ArC*H*CCH_2), 6.45 (1H, br.s. N*H*), 3.93 (6H, s, OCH_3), 3.55 (2H, td, *J* = 6.5, 2.5 Hz, NCH_2CH_2) and 2.93 (2H, t, *J* = 6.5 Hz, NHCH_2CH_2). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ_C 166.4- (*C*=O), 150.8- (Ar*C*O), 148.0- (Ar*C*O), 132.6- (Ar*C*CH_2), 121.3- (Ar*C*(CO)), 110.2+ (Ar*C*HC(CO)), 109.6+ (Ar*C*HCCH_2), 56.1+ (O*C*H_3), 56.0+ (O*C*H_3), 40.4- (N*C*H_2CH_2) and 28.4- (NCH_2*C*H_2). **MS** 208 (75 %, MH^+) and 230 (59 %, MNa^+). **HRMS** *m/z* (+ESI) Found MNa^+ 230.0813, $C_{11}H_{13}NNaO_3$ requires *MNa* 230.0793. **Rf** (100 % EtOAc) 0.2. Spectroscopic data are consistent with those reported by Judd *et al.*[93]

Synthesis of 6,7,8-trimethoxy-3,4-dihydroisoquinolin-1(2H)-one 115



Following **General Procedure 6,** using indanone **92** (340 mg, 1.53 mmol, 1 eq.) and NaN_3 (199 mg, 3.06 mmol, 2 eq.) in TFA (2.5 mL) desired lactam **115** (113 mg, 31 % yield) was isolated as a beige solid. **m.p.** 131-134 ^{o}C [lit.[184] 138 ^{o}C]. **^{1}H NMR** (400 MHz, $CDCl_3$): δ_H 6.49 (1H, s, ArC*H*), 6.09 (1H, br. N*H*), 3.94 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.43 (2H, td, *J* = 6.5, 3.5 Hz, NC$H_2$$CH_2$) and 2.87 (2H, t, *J* = 6.5 Hz, NCH_2CH_2). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 164.4- (C=O), 155.9- (*C*), 155.3- (*C*), 142.0- (*C*), 136.7- (*C*), 115.8- (*C*), 106.0+ (Ar*C*H), 61.8+ (O*C*H_3), 61.1+ (O*C*H_3), 55.9+ (O*C*H_3), 39.8- (N*C*$H_2$$CH_2$) and 30.1- ($NCH_2$*C*$H_2$). **MS** *m/z* (+ESI) 238 (100 %, MH^+) and 260 (29 %, MNa^+). **HRMS** (+ESI) Found MH^+ 238.1133, $C_{12}H_{16}NO_4$ requires *MH* 238.1079 and found MNa^+ 260.0897, $C_{12}H_{15}NNaO_4$ requires *MNa* 260.0899. **IR** ν_{max}(liquid film): 3400 (NH), 2990 (CH) and 1668 (C=O). **Rf** (100 % EtOAc) 0.1.

Synthesis of 2,3,4,5-tetrahydroazepino[3,4-*b*]indol-1(*10H*)-one 116



96 **116**

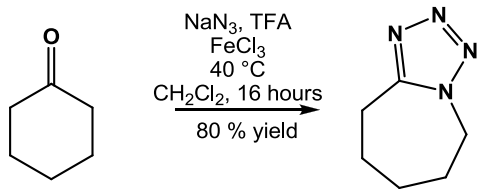
Compound **96** (656 mg, 3.54 mmol, 1 eq.), NaN_3 (460 mg, 7.08 mmol, 2 eq.) and TFA (10 mL) were heated at 100 ^oC for 18 hours in a sealed pressure tube. On cooling, the reaction mixture was transferred to a flask using $CHCl_3$ and the solvent was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], the desired lactam **116** (167 mg, 24 % yield) was isolated as a beige solid, **m.p.** 226-229 ^oC [lit.[93] 227-230 ^oC]. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 9.30 (1H, br. s. N*H*), 7.59 (1H, d, *J* = 8.0 Hz, ArC(3)*H* or ArC(6)*H*), 7.40 (1H, d, *J* = 8.0 Hz, ArC(3)*H* or ArC(6)*H*), 7.32 (1H, td, *J* = 8.0, 1.0 Hz, ArC(4)*H* or ArC(5)*H*), 7.13 (1H, td, *J* = 8.0, 1.0 Hz, ArC(4)*H* or ArC(5)*H*), 7.02 (1H, br. s. NH), 3.51-3.48 (2H, m, CH_2), 3.14 (2H, t, *J* = 6.0 Hz, CH_2) and 2.22-2.17 (2H, m, CH_2). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 165.9- (C=O), 136.0- (*C*), 128.0- (*C*), 125.8- (*C*), 125.5+ (Ar*C*(4)H or Ar*C*(5)H), 120.3+ (Ar*C*(3)H or Ar*C*(6)H), 119.9+ (Ar*C*(4)H or Ar*C*(5)H), 119.9- (*C*), 111.8+ (Ar*C*(3)H or Ar*C*(6)H), 43.1- (*C*H_2), 26.5- (*C*H_2) and 25.8- (CH_2). **MS** *m/z* (+ESI) 201 (100 %, MH^+) and 223 (50 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 223.0846, $C_{12}H_{12}N_2NaO$ requires *MNa* 223.0847. **Rf** (50 % EtOAc in light petroleum (b.p. 40-60 ^oC) 0.3. Spectroscopic data are consistent with Judd *et al.*[93]

7.4.3. Tetrazoles

General Procedure 7, Schmidt and Tetrazole Reaction

The required indanone (1 eq.) and NaN_3 (4 eq.) in TFA (3 mL/ 1 mmol) were heated at 100 ^oC in a sealed pressure tube for 18 hours. The reaction mixture was cooled and sat. aq. $NaHCO_3$ was added dropwise followed by EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc (x 2). The combined organic layers were washed with brine, dried on Na_2SO_4, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) – EtOAc - MeOH gradient column], the desired lactam was isolated.

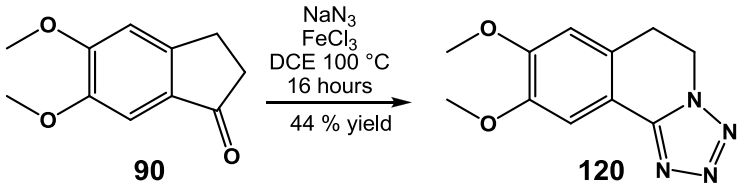
Synthesis of 6,7,8,9-tetrahydro-5H-tetrazolo[1,5-a]azepine 119



117 **119**

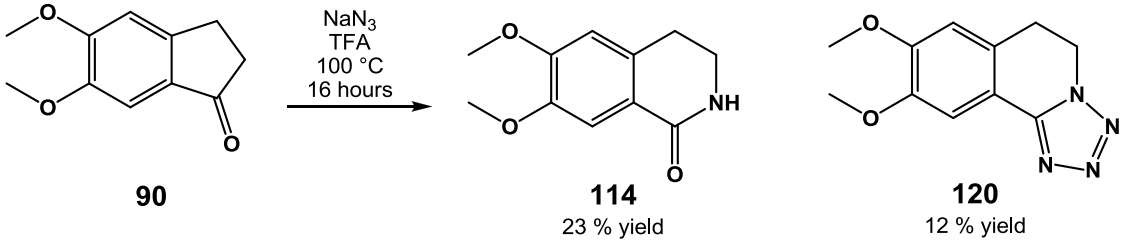
Cyclohexanone **117** (0.21 mL, 2 mmol, 1 eq.), NaN_3 (520 mg, 8 mmol, 4 eq.) and $FeCl_3$ (973 mg, 6 mmol, 3 eq.) in CH_2Cl_2 (10 mL) were heated at 40 ^oC for 16 hours. On cooling the solvent was removed under reduced pressure. 2M $NaOH_{(aq.)}$ (10 mL) and EtOAc (10 mL) were added, the layers separated and the aqueous layer washed with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure to collect 307 mg of yellow oil. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], tetrazole **119** (220 mg, 80 % yield) was isolated as a white solid, **m.p.** 58-61 ^oC [lit.[185] 60 ^oC]. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 4.42-4.39 (2H, m, NCH_2), 3.01-2.98 (2H, m, CCH_2), 1.92-1.86 (2H, m, CH_2), 1.82-1.76 (2H, m, CH_2) and 1.70-1.65 (CH_2). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 156.6- (*C*=N), 49.2- (N*C*H_2), 29.7- (*C*H_2), 27.0- (*C*H_2), 24.5- (*C*H_2) and 24.1 (C*C*H_2). Spectroscopic data are consistent with those reported by Eshghi and Hassankhani.[101]

Synthesis of 8,9-dimethoxy-5,6-dihydrotetrazolo[5,1-a]isoquinoline 120



Indanone **90** (156 mg, 0.81 mmol, 1 eq.) was dissolved in dichloroethane (5 mL). NaN_3 (211 mg, 3.24 mmol, 4 eq.) and $FeCl_3$ (394 mg, 2.43 mmol, 3 eq.) were added and the reaction mixture was heated in a sealed pressure tube at 100 ^{o}C for 16 hours. On cooling, the reaction mixture was transferred to a flask using $CHCl_3$ and the solvent was removed under reduced pressure. EtOAc (30 mL) and 2M $NaOH_{(aq.)}$ (30 mL) were added, the layers were separated and the aqueous layer was washed with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (20 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure to collect 185 mg of yellow solid. After column chromatography [silica, MeOH - CH_2Cl_2 gradient column], the tetrazole **120** (82 mg, 44 % yield) was isolated as a yellow solid, **m.p.** 133-136 ^{o}C. **^{1}H NMR** (400 MHz, $CDCl_3$): δ_H 7.61 (1H, s, ArC*H*C(CN)), 6.84 (1H, s, ArC*H*CCH_2), 4.63 (2H, t, *J* = 6.0 Hz, NC*H*$_2$CH_2), 3.97 (3H, s, OC*H*$_3$), 3.95 (3H, s, OC*H*$_3$) and 3.28 (2H, t, *J* = 6.0 Hz, NCH_2C*H*$_2$). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 151.9- (Ar*C*OCH_3), 151.1- (*C*=N), 149.0- (Ar*C*OCH_3), 127.0- (Ar*C*), 113.6- (Ar*C*), 110.9+ (Ar*C*HCCH_2), 108.2+ (Ar*C*HC(CN)), 53.3+ (OCH_3), 56.1+ (OCH_3), 43.9- (N*C*H$_2$CH_2) and 27.9- (NCH_2*C*H$_2$). **MS** *m/z* (+ESI) 233 (72 %, MH^+) and 255 (100 %, MNa^+). **HRMS** (+ESI) Found MH^+ 233.1022, $C_{11}H_{13}N_4O_2$ requires *MH* 233.1039 (7 ppm). **IR** ν_{max}(liquid film): 2990 (CH) and 1509 (C=N). **Rf** (100 % EtOAc) 0.6. **HMBC** See appendices **8.2.1.**

Synthesis of 6,7-dimethoxy-3,4-dihydroisoquinolin-1(2H)-one 114 and 8,9-dimethoxy-5,6-dihydrotetrazolo[5,1-a]isoquinoline 120

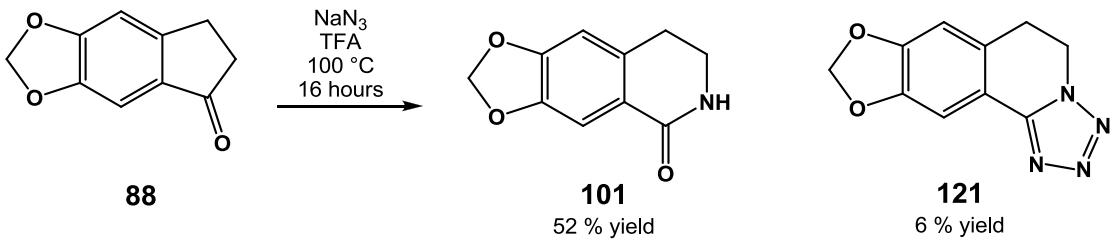


Following **General Procedure 7**, using indanone **90** (154 mg, 0.80 mmol, 1 eq.) and NaN_3 (208 mg, 3.20 mmol, 4 eq.) in TFA (2 mL) lactam **114** (38 mg, 23 % yield) and tetrazole **120** (23 mg, 12 % yield) were isolated as yellow solids.

Lactam 114 Spectroscopic data are consistent with those previously reported for lactam **114** (Page 170).

Tetrazole 120 m.p. 133-136 ^{o}C. **^{1}H NMR** (400 MHz, $CDCl_3$): **^{1}H NMR** (400 MHz, $CDCl_3$): δ_H 7.61 (1H, s, ArC*H*C(CN)), 6.84 (1H, s, ArC*H*CCH_2), 4.63 (2H, t, *J* = 6.0 Hz, NC*H*$_2$CH_2), 3.97 (3H, s, OC*H*$_3$), 3.95 (3H, s, OC*H*$_3$) and 3.28 (2H, t, *J* = 6.0 Hz, NCH_2C*H*$_2$). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 151.9- (Ar*C*OCH_3), 151.1- (*C*=N), 149.0- (Ar*C*OCH_3), 127.0- (Ar*C*), 113.6- (Ar*C*), 110.9+ (Ar*C*HCCH_2), 108.2+ (Ar*C*HC(CN)), 53.3+ (OCH_3), 56.1+ (OCH_3), 43.9- (N*C*H_2CH_2) and 27.9- (NCH_2*C*H_2). **MS** *m/z* (+ESI) 233 (72 %, MH^+) and 255 (100 %, MNa^+). **HRMS** (+ESI) Found MH^+ 233.1022, $C_{11}H_{13}N_4O_2$ requires *MH* 233.1039 (7 ppm). **IR** ν_{max}(liquid film): 2990 (CH) and 1509 (C=N). **Rf** (100 % EtOAc) 0.6. **HMBC** See appendices **8.2.1.**

Synthesis of 6,7-dimethoxy-3,4-dihydroisoquinolin-1(2H)-one 101 and 5,6-dihydro-[1,3]dioxolo[4,5-g]tetrazolo[5,1-a]isoquinoline 121



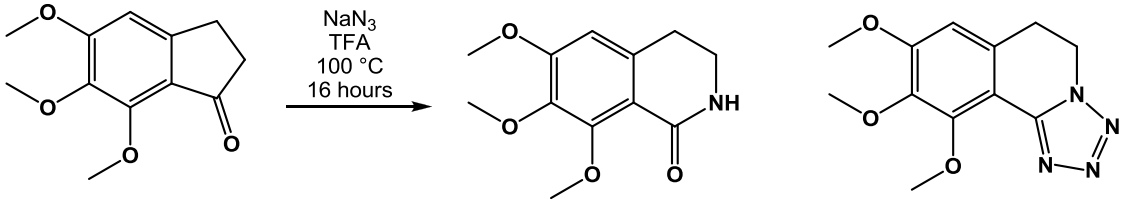
88 **101** 52 % yield **121** 6 % yield

Following **General Procedure 7**, using indanone **88** (141 mg, 0.80 mmol, 1 eq.) and NaN_3 (208 mg, 3.20 mmol, 4 eq.) in TFA (2 mL) lactam **101** (80 mg, 52 % yield) as a yellow solid and tetrazole **121** (11 mg, 6 % yield) as a beige solid, were isolated.

Lactam 101 Spectroscopic data are consistent with those previously reported for lactam **101** (Page 169).

Tetrazole 121 m.p. 147-150 ^{o}C. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.56 (1H, s, ArC*H*C(CN)), 6.81 (1H, s, ArC*H*CCH_2), 6.06 (2H, s, OCH_2O), 4.59 (2H, t, *J* = 7.0 Hz, NCH_2CH_2) and 3.24 (2H, t, *J* = 7.0 Hz, NCH_2CH_2). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 151.1- (*C*=*N*), 150.8- (*C*), 147.7- (*C*), 128.8- (*C*), 114.8- (C) 108.6+ (Ar*C*HCCH_2), 105.9+ (Ar*C*HC(CN)), 101.9- (OCH_2O), 43.6- (N*C*H_2CH_2) and 28.3- (NCH_2*C*H_2). **MS** *m/z* (ESI+) 217 (100 %, MH^+) and 239 (73 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 239.0568, $C_{10}H_8N_4NaO_2$ requires *MNa* 239.0545 (10 ppm). **IR** ν_{max}(liquid film): 2985 (CH) and 1505 (C=N). **Rf** (100 % EtOAc) 0.6.

Synthesis of 6,7,8-trimethoxy-3,4-dihydroisoquinolin-1(2H)-one 115 and 8,9,10-trimethoxy-5,6-dihydrotetrazolo[5,1-a]isoquinoline 122



92 | **115** 16 % yield | **122** 10 % yield

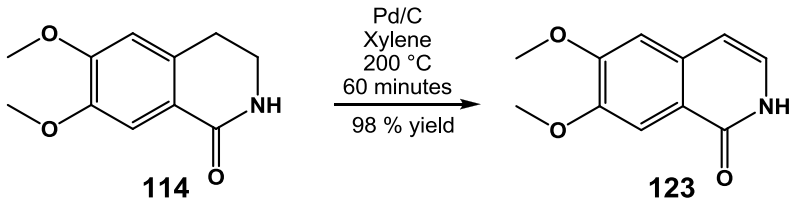
Following **General Procedure 7**, using indanone **92** (154 mg, 0.80 mmol, 1 eq.) and NaN_3 (208 mg, 3.20 mmol, 4 eq.) in TFA (2 mL), lactam **115** (38 mg, 23% yield) and tetrazole **122** (23 mg, 12 % yield) were isolated as beige solids.

Lactam 115 Spectroscopic data are consistent with those previously reported for lactam **115** (Page 171).

Tetrazole 122 m.p. 115-117 ^{o}C. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 6.67 (1H, s, ArC*H*), 4.59 (2H, t, *J* = 7.0 Hz, NC*H*$_2$CH$_2$), 4.07 (3H, s, OC*H*$_3$), 3.93 (3H, s, OC*H*$_3$), 3.91 (3H, s, OC*H*$_3$) and 3.22 (2H, t, *J* = 7.0 Hz, NCH$_2$C*H*$_2$). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 156.3- (*C*=N), 152.5- (*C*), 149.2- (*C*), 142.4- (*C*), 130.6- (*C*), 109.0- (*C*), 107.5+ (Ar*C*H), 61.6+ (O*C*H$_3$), 61.1+ (O*C*H$_3$), 56.2+ (O*C*H$_3$), 43.5- (N*C*H$_2$CH$_2$) and 28.9- (NCH$_2$*C*H$_2$). **MS** *m/z* (ESI+) 263 (100 %, MH$^+$) and 285 (30 %, MNa$^+$). **HRMS** (+ESI) Found MNa$^+$ 285.0996, $C_{12}H_{14}N_4NaO_3$ requires *MNa* 285.0964 (11 ppm). **IR** ν_{max}(liquid film): 2990 (CH) and 1503 (C=N). **Rf** (100 % EtOAc) 0.7.

7.4.4. Oxidation Reactions

Synthesis of 6,7-dimethoxyisoquinolin-1(2H)-one 123



Method A - Optimised Method

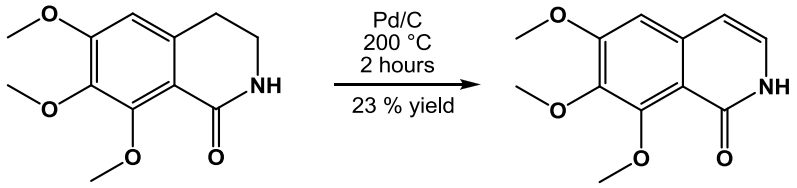
Following a procedure adapted from Dufour and Kirsch,[107] Pd/C (10 % wt) (100 mg, 0.1 mmol, 10 mol %) was added to lactam **114** (207 mg, 1 mmol, 1 eq.) in xylene (5 mL). The reaction vessel was degassed and placed in a microwave at 200 ^{o}C for 30 minutes, 300 W power. MeOH (10 mL) and silica were added and the reaction mixture was heated at reflux for 1 hour. The reaction mixture was concentrated under reduced pressure and dry loaded on a column. After column chromatography [silica, MeOH - CH_2Cl_2 gradient column], product **123** (201 mg, 98 % yield) was isolated as a white solid.

Method B

Following a procedure reported by Mcnulty and Still,[109] Pd/C (10 % wt) (10 mg) and lactam **114** (20 mg, 10 mmol, 1 eq.) were ground together to a fine powder. The solid was transferred to a flask, flushed with N_2 and the reaction mixture was heated to 200 ^{o}C for 1 hour. On cooling the reaction mixture was flushed through a pad of celite using hot MeOH. The solvent was removed under reduced pressure to collect **123** (83 mg, 81 % yield) as a white solid.

m.p. >220 ^{o}C [lit.[186] 227-230 ^{o}C]. **^{1}H NMR** (400 MHz, $CDCl_3$): δ_H 11.35 (1H, br.s. N*H*), 7.79 (1H, s, ArC*H*(C=O)), 7.09 (1H, d, *J* = 7.0 Hz, NHC*H*CH), 6.91 (1H, s, ArC*H*), 6.48 (1H, d, *J* = 7.0 Hz, NCHC*H*) 4.02 (3H, s, OCH_3) and 4.00 (3H, s, OCH_3). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 163.4- (*C*=O), 153.8- (*C*), 149.4- (*C*), 133.8- (*C*), 126.3+ (NCHCH), 120.1- (*C*), 107.2+ (Ar*C*H(C=O)), 106.3+ (Ar*C*H), 106.2+ (NCH*C*H), 56.2+ (O*C*H_3) and 56.1+ (O*C*H_3). **MS** *m/z* (+ESI) 206 (100 %, MH^+) and 228 (86 %, MNa^+). **HRMS** (+ESI) Found MH^+ 206.0817, $C_{11}H_{12}NO_3$ requires *MH* 206.0817 and found MNa^+ 228.0626, $C_{11}H_{11}NNaO_3$ requires *MNa* 228.0637. **IR** ν_{max}(liquid film): 3420 (NH), 2995 (CH) and 1655 (C=O). **Rf** (100 % EtOAc) 0.2.

Synthesis of 6,7,8-trimethoxyisoquinolin-1(2H)-one 124



115 **124**

Following a procedure reported by Mcnulty and Still,[109] Pd/C (10 % wt) (13 mg) and lactam **115** (30 mg, 0.13 mmol, 1 eq.) were ground together to a fine powder. The solid was transferred to a flask, flushed with N_2 and the reaction mixture was heated to 200 ^{o}C for 2 hours. On cooling the reaction mixture was flushed through a pad of celite using hot MeOH. The solvent was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc - MeOH gradient column], **124** (7 mg, 23 % yield) was isolated as a white solid, **m.p.** 193-196 ^{o}C [lit.[187] 198 ^{o}C]. **^{1}H NMR** (400 MHz, $CDCl_3$): δ_H 9.15 (1H, br.s. N*H*), 6.96 (1H, d, *J* = 7.0 Hz, NHC*H*CH), 6.69 (1H, s, ArC*H*), 6.32 (1H, d, *J* = 7.0 Hz, NHCHC*H*), 3.99 (3H, s, OCH_3), 3.95 (3H, s, OCH_3) and 3.93 (3H, s, OCH_3). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 161.1- (*C*=O), 157.4- (*C*), 154.5- (*C*), 142.2- (*C*), 136.9- (*C*), 127.2+ (N*C*HCH), 114.7- (*C*), 105.8+ (NCH*C*H), 103.0+ (Ar*C*H), 62.1+ (O*C*H_3), 61.5+ (O*C*H_3) and 56.0+ (O*C*H_3). **MS** *m/z* (+ESI) 236 (100 %, MH^+) and 258 (62 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 258.0730, $C_{12}H_{13}NNaO_4$ requires *MNa* 258.0742. **IR** ν_{max}(liquid film): 3450 (NH), 2900 (CH), 1667 (C=O) and 1595 (C=C). **Rf** (100 % EtOAc) 0.2.

7.5. Chapter Four - A/B/C Analogues of Dihydroisoquinolinones

7.5.1. Intermolecular Indanone Synthesis

General Procedure 8, Indanone Synthesis - Route 1

Cyclohexene-1-carboxylic acid and 1,3-benzodioxole in TFAA/ TFA were heated at 100 ^{o}C in a sealed pressure tube. On cooling, the reaction mixture was transferred to a flask using $CHCl_3$ and the solvent was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60^{o}C)-EtOAc gradient column], the desired indanone was isolated.

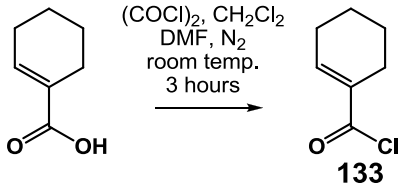
General Procedure 9, Indanone Synthesis - Route 1

Cyclohexene-1-carboxylic acid and 1,3-benzodioxole in TFAA/ TFA were heated at 100 ^{o}C for 4 hours in a sealed pressure tube. On cooling, the reaction mixture was transferred to a flask using $CHCl_3$ and the solvent was removed under reduced pressure. Sat. aqueous $NH_4^+Cl^-$ (30 mL) and EtOAc (30 mL) were added, the layers were separated and the aqueous layer was washed with EtOAc (2 x 30 mL). The combined organic layers were washed with brine (30 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure. After column chromatography [silica, CH_2Cl_2 - MeOH gradient column], the desired indanone was isolated.

General Procedure 10, Indanone Synthesis - Route 2

Piperonylic acid (1 eq.) and cyclohexene/ cyclopentene (1.0, 1.1 or 2.0 eq.) in (TFAA/ TFA) were heated in a sealed pressure tube at 100 ^{o}C. On cooling, $CHCl_3$ was added and the solvent was removed under reduced pressure. EtOAc (5 mL) and H_2O (5 mL) were added and the reaction mixture stirred at room temperature for 22 hours. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (20 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the desired indanone was isolated.

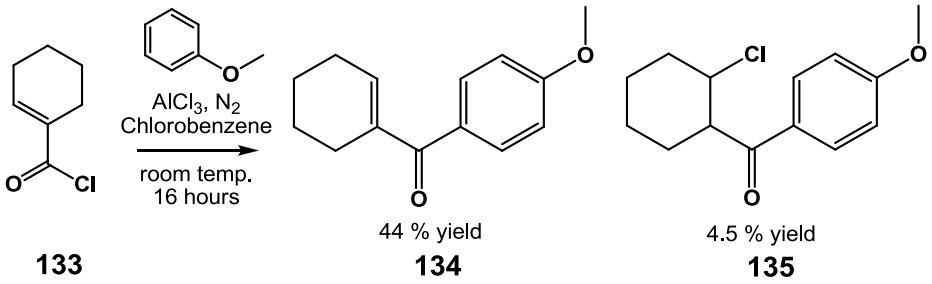
Synthesis of cyclohex-1-ene carbonyl chloride 133



133

Following a procedure reported by Sibi *et al.*,[118] a solution of cyclohexene-1-carboxylic acid (378 mg, 3 mmol, 1 eq.) in CH_2Cl_2 (4 mL) at 0 ^oC under a N_2 atmosphere was added oxalyl choride (0.30 mL, 3.3 mmol, 1.1 eq.) followed by DMF (1 drop). After 10 minutes, the reaction mixture was warmed to room temperature and stirred for 3 hours. The solvent was removed under reduced pressure and the compound was used without purification in the next reaction.

Synthesis of cyclohexenyl(4-methoxyphenyl)methanone 134 and (2-chloro-cyclohexyl)(4-methoxyphenyl)methanone 135



133 **134** 44 % yield **135** 4.5 % yield

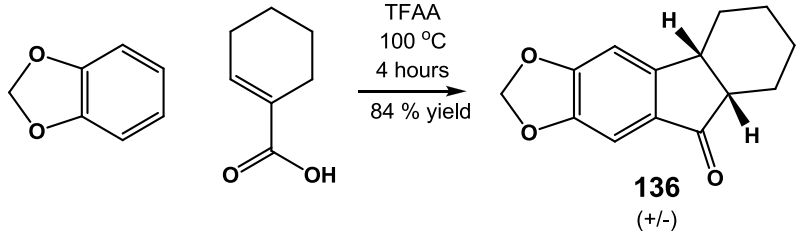
Following the procedure reported by Yin *et al* but at room temperature,[116] to a rapidly stirred solution of acid chloride **133**, (433 mg, 2.99 mmol, 1.1 eq.) and anisole (0.3 mL, 2.72 mmol, 1 eq.) in anhydrous chlorobenzene (6 mL) was added anhydrous $AlCl_3$ (1.09 g, 8.16 mmol, 3 eq.) at room temperature. This was placed under a N_2 atmosphere and stirred for 16 hours. 12M $HCl_{(aq.)}$ (10 mL) was added, followed by ice (50 mL). The mixture was stirred until all the ice had melted and the resulting mixture was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with 2M $HCl_{(aq.)}$ (10 mL), H_2O (10 mL), aq. $NaHCO_3$ (10 mL) and H_2O (10 mL), dried on Na_2SO_4, filtered and the solvent was removed under reduced pressure to collect crude brown oil (1.16 g). After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], the compounds **134** (260 mg, 44 % yield) and **135** (31 mg, 4.5 % yield) were isolated as yellow oils.

134 **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.68 (2H, d, *J* = 8.5 Hz, ArC(2)*H*), 6.91 (2H, d, *J* = 8.5 Hz, ArC(3)*H*), 6.48 (1H, m, alkene C*H*), 3.84 (3H, s, OCH_3), 2.41-2.38 (2H, m, CHCH_2), 2.26-2.22 (2H, m, CH_2) and 1.74-1.65 (4H, m, CH_2CH_2). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 197.1- (*C*=O), 162.4- (Ar*C*(4)), 141.5+ (alkene *C*H), 138.6- ((CO)*C*CH), 131.5+ (Ar*C*(2)H), 130.9- (Ar*C*(1)), 113.3+ (Ar*C*(3)H), 55.4+ (O*C*H_3), 25.9- (*C*H_2), 24.3- (CH*C*H_2), 22.1- (*C*H_2) and 21.7- (*C*H_2). **MS** *m/z* (+ESI) 217 (89 %, MH^+) and 239 (100 %, MNa^+). **HRMS** (+ESI) Found MH^+ 217.1231, $C_{14}H_{17}O_2$ requires *MH* 217.1229 and found MNa^+ 239.1046, $C_{14}H_{16}NaO_2$ requires *MNa* 239.1048. **IR** ν_{max}(liquid film): 2859 (CH), 1638 (C=O), 1600 (C=C) and 1029 (C-O). **Rf** (20 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.5.

135 **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.77 (2H, d, *J* = 8.5 Hz, ArC(2)*H*), 6.87 (2H, d, *J* = 8.5 Hz, ArC(3)*H*), 4.57-4.55 (1H, m, ClC*H*), 3.79 (3H, s, OCH_3), 3.50-3.45 (1H, m, (CO)C*H*) and 2.12-1.18 (8H, m, CH_2CH_2CH_2CH_2). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 198.2- (*C*=O), 163.3- (ArC(4)), 130.3+ (Ar*C*(2)H), 129.1- (Ar*C*(1)), 113.9+ (Ar*C*(3)H), 60.5+ (Cl*C*H), 55.5+ (O*C*H_3), 49.1+ ((CO)*C*H), 34.3- (*C*H_2), 24.2- (*C*H_2), 22.1- (*C*H_2), 20.2- (*C*H_2). **MS** *m/z* (+ESI) 253 (17 %, MH^+), 275 (100 %, MNa^+ (^{35}Cl)) and 277 (30 %, MNa^+ (^{37}Cl)). **HRMS** (+ESI) Found MH^+ 253.0986, $C_{14}H_{18}{}^{35}ClO_2$ requires *MH* 253.0995 and found MNa^+ 275.0805, $C_{14}H_{17}{}^{35}ClNaO_2$ requires *MNa* 275.0815. **IR** ν_{max}(liquid film): 2937 (CH), 1374 (C=O), 1599 (C=C) and 1023 (C-O). **Rf** (20 % EtOAc in light petroleum (b.p. 40-60 ^{o}C) 0.5.

Synthesis of (4bR,8aS)-6,7,8,8a-tetrahydro-4bH-fluoreno[2,3-*d*][1,3]dioxol-9(*5H*)one 136

Route 1



Method 1A

Following **General Procedure 8**, cyclohexene-1-carboxylic acid (126 mg, 1 mmol, 1 eq.) and 1,3-benzodioxole (0.11 mL, 1 mmol, 1 eq.) in TFAA: TFA (0.5 mL: 1.0 mL) at 100 ^{o}C for 1 hour afforded indanone **136** (151 mg, 66 % yield) as a yellow solid.

Method 1B

Following **General Procedure 8**, cyclohexene-1-carboxylic acid (126 mg, 1 mmol, 1 eq.) and 1,3-benzodioxole (0.11 mL, 1 mmol, 1 eq.) in TFAA: TFA (0.5 mL: 1.0 mL) at 100 ^{o}C for 3.5 hours afforded indanone **136** (188 mg, 82 % yield) as a yellow solid.

Method 1C

Following **General Procedure 8**, cyclohexene-1-carboxylic acid (126 mg, 1 mmol, 1 eq.) and 1,3-benzodioxole (0.11 mL, 1 mmol, 1 eq.) in TFAA: TFA (0.5 mL: 1.0 mL) at 100 ^{o}C for 6 hours afforded indanone **136** (166 mg, 72 % yield) as a yellow solid.

Method 1D

Following **General Procedure 8**, cyclohexene-1-carboxylic acid (126 mg, 1 mmol, 1 eq.) and 1,3-benzodioxole (0.13 mL, 1.1 mmol, 1.1 eq.) in TFAA: TFA (0.5 mL: 1.0 mL) at 100 ^{o}C for 17 hours afforded indanone **136** (129 mg, 56 % yield) as a yellow solid.

Method 1E

Following **General Procedure 9**, cyclohexene-1-carboxylic acid (252 mg, 2 mmol, 1 eq.) and 1,3-benzodioxole (0.46 mL, 4 mmol, 2 eq.) in TFAA: TFA (1.0 mL: 2.0 mL) at 100 ^{o}C for 4 hours afforded indanone **136** (270 mg, 59 % yield) as a yellow solid.

Method 1F

Following **General Procedure 9**, cyclohexene-1-carboxylic acid (126 mg, 1 mmol, 1 eq.) and 1,3-benzodioxole (0.23 mL, 2 mmol, 2 eq.) in TFAA (0.49 mL, 3.5 mmol, 3.5 eq.) at 100 ^{o}C for 4 hours afforded indanone **136** (172 mg, 75 % yield) as a yellow solid.

Method 1G

Following a procedure reported by Luke *et al*,[120] cyclohexene-1-carboxylic acid (126 mg, 1 mmol, 1 eq.), 1,3-benzodioxole (0.23 mL, 2 mmol, 2 eq.) and TFAA (0.56 mL, 4 mmol, 4 eq.) were added to a pressure tube. The reaction mixture was cooled to 0 ^{o}C and H_3PO_4 (0.06 mL, 1 mmol, 1 eq.) was added. The tube was sealed and heated at 100 ^{o}C for 4 hours. On cooling to 0 ^{o}C, the reaction mixture was diluted with H_2O (5 mL) and the pH was adjusted to ~ pH 9 by the careful addition of 10M $NaOH_{(aq.)}$. EtOAc (10 mL) was added and the reaction mixture was stirred for 10 minutes at room temperature, the layers were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (20 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the indanone **136** (130 mg, 57 % yield) was isolated as a yellow solid.

Method 1H

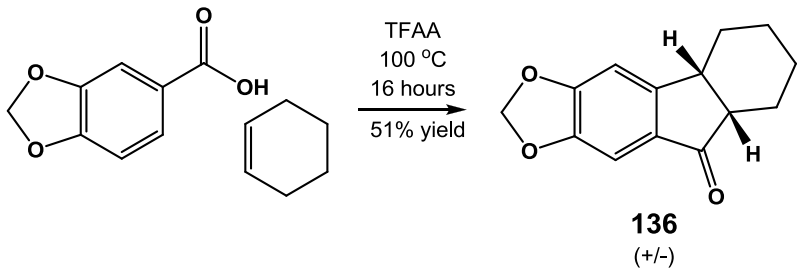
Cyclohexene-1-carboxylic acid (126 mg, 1 mmol, 1 eq.), 1,3-benzodioxole (0.23 mL, 2 mmol, 2 eq.) and TFAA (0.56 mL, 4 mmol, 4 eq.) were added to a pressure tube. The reaction mixture was cooled to 0 ^{o}C and H_2SO_4 (0.05 mL, 1 mmol, 1 eq.) was added and the reaction mixture was heated at 100 ^{o}C for 4 hours in a sealed pressure tube. On cooling to 0 ^{o}C, the reaction mixture was diluted with H_2O (5 mL) and the pH adjusted to ~ pH 9 by the careful addition of 10M $NaOH_{(aq.)}$. EtOAc (10 mL) was added and the reaction mixture was stirred for 10 minutes at room temperature, the layers were separated and the aqueous layer extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (20 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the indanone **136** was isolated (72 mg, 31 % yield) as a yellow solid.

Method 1I - Optimised Method

Following **General Procedure 8**, cyclohexene-1-carboxylic acid (505 mg, 4 mmol, 1 eq.) and 1,3-benzodioxole (0.92 mL, 8 mmol, 2 eq.) in TFAA (1.95 mL, 14 mmol, 3.5 eq.) were

heated at 100 ^{o}C for 4 hours. Using MeOH to transfer the reaction mixture, indanone **136** (774 mg, **84 % yield**) was isolated as a yellow solid.

Route 2



136
(+/-)

Method 2A

Following **General Procedure 10**, piperonylic acid on a 1 mmol scale and cyclohexene (1 eq.) in TFAA (3.5 eq.) at 100 ^{o}C for 4 hours, afforded indanone **136** (81 mg, 35 % yield) as a yellow solid.

Method 2B

Following **General Procedure 10**, piperonylic acid on a 1 mmol scale and cyclohexene (1.1 eq.) in TFAA (3.5 eq.) at 100 ^{o}C for 6 hours, afforded indanone **136** (32 mg, 14 % yield) as a yellow solid.

Method 2C - Optimised Method

Following **General Procedure 10**, piperonylic acid on a 1 mmol scale and cyclohexene (1.1 eq.) in TFAA (3.5 eq.) at 100 ^{o}C for 16 hours, afforded indanone **136** (118 mg, **51 % yield**) as a yellow solid.

Method 2D

Following **General Procedure 10**, piperonylic acid on a 2 mmol scale and cyclohexene (2 eq.) in TFAA (3.5 eq.) at 100 ^{o}C for 17 hours, afforded indanone **136** (215 mg, 47 % yield) as a yellow solid.

Method 2E

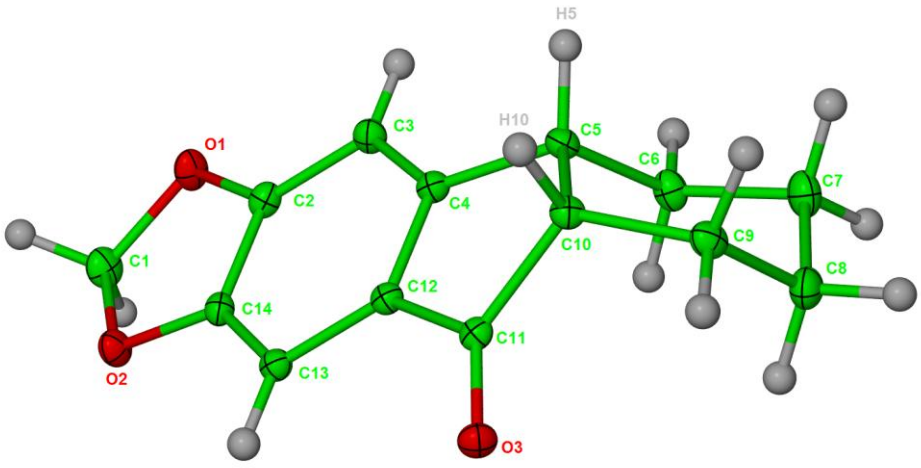
Following **General Procedure 10**, piperonylic acid on a 2 mmol scale and cyclohexene (1.1 eq.) in TFAA (2 eq.) and TFA (1 mL) at 100 ^{o}C for 17 hours, afforded indanone **136** (47 mg, 10 % yield) as a yellow solid.

Method 2F

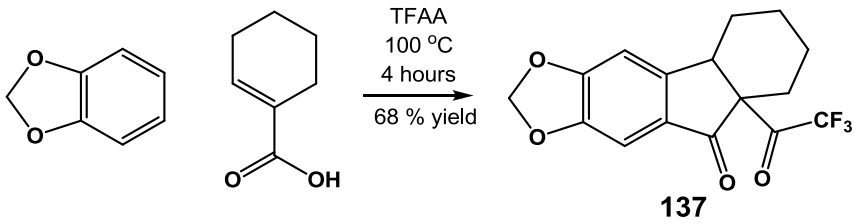
Following **General Procedure 10**, piperonylic acid on a 2 mmol scale and cyclohexene (1.1 eq.) in TFAA (1.1 eq.) and TFA (1 mL) at 100 ^{o}C for 16 hours, afforded indanone **136** (81 mg, 18 % yield) as a yellow solid.

136 m.p. 113-115 ^{o}C. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.11 (1H, s, ArC*H*C(C=O)), 6.83 (1H, s, ArC*H*CCH), 6.06 (2H, m, OC*H*$_2$O), 3.26 (1H, ddd, *J* = 16.0, 9.0, 7.0 Hz, (CO)C*H*CH), 2.74 (1H, dt, *J* = 12.0, 7.0 Hz, (CO)CHC*H*), 2.09-1.98 (2H, m, C*H*$_2$), 1.79-1.70 (1H, m, C*H*$_2$), 1.57-1.33 (3H, m, C*H*$_2$C*H*$_2$) and 1.30-1.16 (2H, m, C*H*$_2$). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 206.1- (C=O), 156.0- (Ar*C*), 153.8- (Ar*C*), 148.0- (Ar*C*), 130.1- (Ar*C*), 104.6+ (Ar*C*HCCH), 102.8+ (Ar*C*HC(C=O)), 102.0- (O*C*H$_2$O), 48.5+ ((CO)CH*C*H), 38.5+ ((CO)*C*HCH), 30.7- (*C*H$_2$), 23.2- (*C*H$_2$), 22.1- (*C*H$_2$) and 21.9- (*C*H$_2$). **MS** (+ESI) 231 (100 %, MH$^+$) and 253 (85 %, MNa$^+$). **HRMS** *m/z* (+ESI) Found MH$^+$ 231.1004, $C_{14}H_{15}O_3$ requires *MH*$^+$ 231.1021 (7 ppm). **IR** ν_{max}(liquid film): 3054 (CH), 1693 (C=O) and 1031 (C-O). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.8.

Crystal Structure (See appendices **8.3.1.** for crystal data)



Synthesis of 8a-trifluoroacetyl-6,7,8,8a-tetrahydro-4bH-fluoreno[2,3-*d*][1,3] dioxol-9(*5H*)-one 137

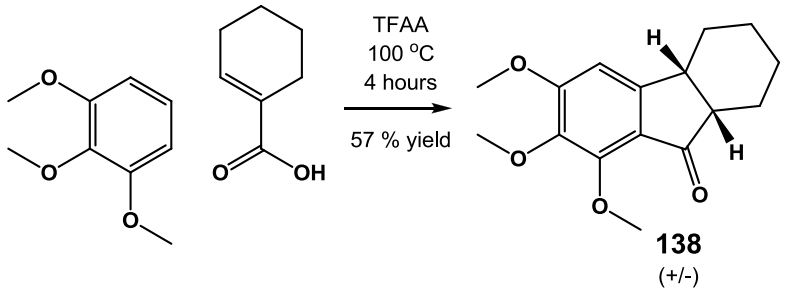


137

Cyclohexene-1-carboxylic acid (505 mg, 4 mmol, 1 eq.) and 1,3-benzodioxole (0.92 mL, 8 mmol, 2 eq.) in TFAA (1.95 mL, 14 mmol, 3.5 eq.) were heated at 100 ^{o}C for 4 hours in a sealed pressure tube. On cooling, the reaction mixture was transferred to a flask using chloroform and the solvent was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the indanone **137** (887 mg, 68 % yield) was isolated as a yellow solid. **^{1}H NMR** (400MHz; $CDCl_3$): δ_H 6.89 (1H, s, ArC*H*C(C=O)), 6.58 (1H, s, ArC*H*CCH), 5.95 (2H, s, OCH_2O), 3.05 (1H, m, C*H*), 2.64-2.59 (1H, m, C*H*-cyclohexane), 2.51-2.47 (1H, m, C*H*-cyclohexane), 2.17 (1H, dt, *J* = 13.0 and 5.0 Hz, C*H*-cyclohexane), 1.99-1.94 (1H, m, C*H*-cyclohexane), 1.90-1.72 (1H, m, C*H*-cyclohexane), 1.52-1.47 (1H, m, C*H*-cyclohexane), 1.30-1.91 (1H, m, C*H*-cyclohexane) and 0.98 (1H, dq, *J* = 13.0 and 3.5 Hz, C*H*-cyclohexane). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 146.8- (*C*=O), 146.3- (*C*=O), 139.0- (*C*), 138.0- (*C*), 135.1- (*C*), 131.0- (*C*), 121.6- (*C*), 116.0- (*C*), 104.7- (Ar*C*HC(C=O)), 101.1- (O*C*H_2O), 98.6+ (Ar*C*HCCH), 46.4+ (*C*H), 32.1- (*C*H_2), 26.6- (*C*H_2), 25.0- (*C*H_2) and 24.7- (*C*H_2). **^{19}F NMR** (400MHz; $CDCl_3$): -74.35 (CF_3). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.9.

Synthesis of 6,7,8-trimethoxy-2,3,4,4a-tetrahydro-1H-fluoren-9(9aH)-one 138

Route 1



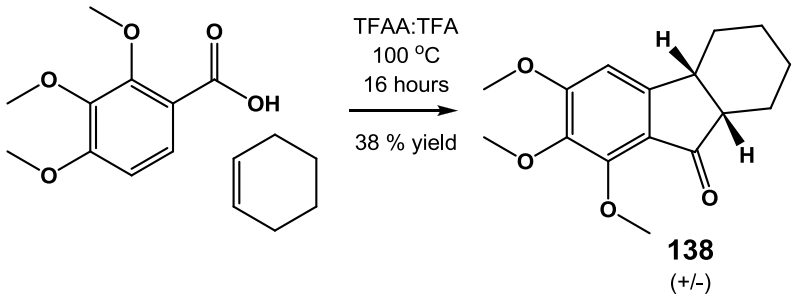
Method 1A

Cyclohexene-1-carboxylic acid (126 mg, 1 mmol, 1 eq.) and 1,2,3-trimethoxybenzene (336 mg, 2 mmol, 2 eq.) in TFAA (0.49 mL, 3.5 mmol, 3.5 eq.) were heated at 100 ^{o}C for 4 hours in a sealed pressure tube. On cooling, the reaction mixture was transferred to a flask using $CHCl_3$ and the solvent was removed under reduced pressure. An initial column [silica, CH_2Cl_2 - MeOH gradient column], followed by a second column [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], afforded indanone **138** (77 mg, 28 % yield) as a white solid.

Method 1B - Optimised Method

Cyclohexene-1-carboxylic acid (126 mg, 1 mmol, 1 eq.) and 1,2,3-trimethoxybenzene (336 mg, 2 mmol, 2 eq.) in TFAA (0.49 mL, 3.5 mmol, 3.5 eq.) were heated at 100 ^{o}C for 4 hours in a sealed pressure tube. On cooling, the reaction mixture was transferred to a flask using $CHCl_3$ and the solvent was removed under reduced pressure. H_2O (5 mL) and EtOAc (5 mL) were added and the reaction mixture was stirred at 20 ^{o}C for 22 hours. The layers were separated and the aqueous layer was washed with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], indanone **138** (156 mg, **57 % yield**) was isolated as a white solid.

Route 2



Method 2A

2,3,4-Trimethoxybenzoic acid (849 mg, 4 mmol, 1 eq.) and cyclohexene (0.45 mL, 4.4 mmol, 1.1 eq.) in TFAA (1.95 mL, 14 mmol, 3.5 eq.) were heated in a pressure tube at 100 ^{o}C for 16 hours. On cooling, the reaction mixture was transferred to a flask using $CHCl_3$ and the solvent was removed under reduced pressure. H_2O (20 mL) and EtOAc (20 mL) were added and the reaction mixture stirred at room temperature for 24 hours. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$ (20 mL), brine (20 mL), dried on $MgSO_4$, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the indanone **138** (142 mg, 13 % yield) was isolated as a light beige solid.

Method 2B

2,3,4-Trimethoxybenzoic acid (424 mg, 2 mmol, 1 eq.) and cyclohexene (0.22 mL, 2.2 mmol, 1.1 eq.) in TFAA (0.97 mL, 7 mmol, 3.5 eq.) were heated in a pressure tube at 100 ^{o}C for 18 hours. On cooling, the reaction mixture was transferred to a flask using $CHCl_3$ and the solvent was removed under reduced pressure. Saturated aq. $NaHCO_3$ (20 mL) and THF (2 mL) were added and the reaction mixture stirred at room temperature for 22 hours. H_2O (20 mL) and EtOAc (20 mL) were added, the layers were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (20 mL), dried on $MgSO_4$, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the indanone **138** was isolated (89 mg, 16 % yield) as a light beige solid.

Method 2C

2,3,4-Trimethoxybenzoic acid (424 mg, 2 mmol, 1 eq.) and cyclohexene (0.22 mL, 2.2 mmol, 1.1 eq.) in TFAA (0.97 mL, 7 mmol, 3.5 eq.) were heated in a pressure tube at 100 ^{o}C for 6 hours. On cooling, the reaction mixture was tranferred to a flask using $CHCl_3$ and the solvent was removed under reduced pressure. Aqueous $NaHCO_3$ (20 mL) and THF (2 mL) were added and the reaction mixture stirred at room temperature for 16 hours. H_2O (20 mL) and EtOAc (20 mL) were added, the layers were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (20 mL), dried on $MgSO_4$, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60^{o}C) EtOAc gradient column], the indanone **138** was isolated (50 mg, 9 % yield) as a light beige solid.

Method 2D

2,3,4-Trimethoxybenzoic acid (212 mg, 1 mmol, 1 eq.) and cyclohexene (0.11 mL, 1.1 mmol, 1.1 eq.) in TFAA (0.97 mL, 7 mmol, 3.5 eq.) were heated in a pressure tube at 100 ^{o}C for 24 hours. On cooling, the reaction mixture was transferred to a flask using $CHCl_3$ and the solvent was removed under reduced pressure. Sat. aq. $NaHCO_3$ (10 mL) and THF (2 mL) were added and the reaction mixture stirred at room temperature for 16 hours. H_2O (10 mL) and EtOAc (10 mL) were added, the layers were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (20 mL), dried on $MgSO_4$, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the indanone **138** was isolated (20 mg, 7 % yield) as a light beige solid.

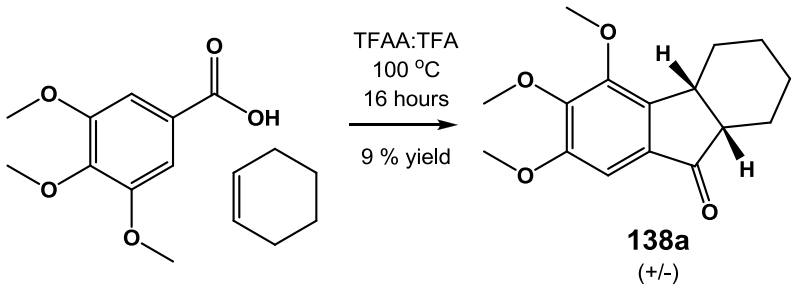
Method 2E - Optimised Method

2,3,4-Trimethoxybenzoic acid (212 mg, 1 mmol, 1 eq.) and cyclohexene (0.20 mL, 2 mmol, 2 eq.) in TFAA (0.15 mL, 1.1 mmol, 1.1 eq.) and TFA (0.5 mL) were heated in a pressure tube at 100 ^{o}C for 16 hours. On cooling, the reaction mixture was tranferred to a flask using $CHCl_3$ and the solvent was removed under reduced pressure. Sat. aq. $NaHCO_3$ (10 mL) and THF (1 mL) were added and the reaction mixture stirred at room temperature for 6 hours. H_2O (10 mL) and EtOAc (20 mL) were added, the layers were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (20 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure. After

column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the indanone **138** was isolated (104 mg, 38 % yield) as a light beige solid.

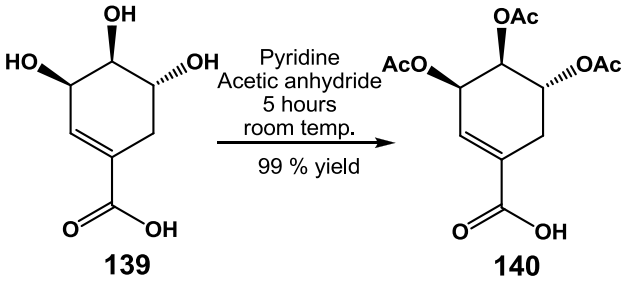
138 m.p. 89-91 ^{o}C. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 6.64 (1H, s, ArC*H*), 4.04 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.23 (1H, dt, *J* = 9.0, 6.5 Hz, COC*H*CH), 2.70 (1H, td, *J* = 7.0, 5.0 Hz, COCHC*H*), 2.09-2.02 (2H, m, CH_2) and 1.74-1.16 (6H, m, CH_2). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 203.9- (*C*=O), 159.3- (*C*), 156.3- (*C*), 151.7- (*C*), 140.5- (*C*), 120.9- (*C*), 102.3+ (Ar*C*H), 61.9+ (*C*H), 61.3+ (*C*H), 56.2+ (O*C*H_3), 48.9+ (O*C*H_3), 38.5+ (O*C*H_3), 31.2- (*C*H_2), 23.2- (*C*H_2), 22.5- (*C*H_2), 22.2- (*C*H_2). **MS** *m/z* (+ESI) 277 (53 %, MH^+) and 299 (18 %, MNa^+). **HRMS** (+ESI) Found MH^+ 277.1427, $C_{16}H_{21}O_4$ requires *MH* 277.1440 and found MNa^+ 299.1248, $C_{16}H_{20}NaO_4$ requires *MNa* 299.1259. **IR** ν_{max}(liquid film): 3054 (CH) and 1702 (C=O). **Rf** (40 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.4.

Synthesis of (4aR,9aS)-5,6,7-trimethoxy-2,3,4,4a-tetrahydro-1H-fluoren-9(9aH)-one 138a



3,4,5-Trimethoxybenzoic acid (424 mg, 2 mmol, 1 eq.) and cyclohexene (0.41 mL, 4 mmol, 2 eq.) in TFAA (0.31 mL, 2.2 mmol, 1.1 eq.) and TFA (1 mL) were heated in a pressure tube at 100 ^{o}C for 16 hours. On cooling, the reaction mixture was transferred to a flask using $CHCl_3$ and the solvent was removed under reduced pressure. Sat. aq. $NaHCO_3$ (10 mL) and THF (1 mL) were added and the reaction mixture stirred at room temperature for 6 hours. H_2O (10 mL) and EtOAc (20 mL) were added, the layers were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic layers were washed with brine (20 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the indanone **138a** was isolated (50 mg, 9 % yield) as a white solid, **m.p.** 72-75 ^{o}C. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.03 (1H, s, ArC*H*), 3.96 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.43 (1H, dt, *J* = 13.0, 7.0 Hz, COC*H*CH), 2.67 (1H, td, *J* = 7.0, 4.0 Hz, COCHC*H*), 2.28-2.21 (2H, m, CH_2), 1.70-1.65 (1H, m, CH_2), 1.62-1.53 (2H, m, CH_2) and 1.37-0.93 (2H, m, CH_2). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 206.6- (*C*=O), 153.9- (*C*), 150.0- (*C*), 147.5- (*C*), 144.9- (*C*), 131.0- (*C*), 101.1+ (Ar*C*H), 60.9+ (O*C*H$_3$), 60.9+ (O*C*H$_3$), 56.1+ (O*C*H$_3$), 48.4+ (COCH*C*H), 36.3+ (CO*C*HCH), 31.1- (*C*H$_2$), 22.8- (*C*H$_2$), 22.8- (*C*H$_2$) and 22.1- (*C*H$_2$). **MS** *m/z* (+ESI) 277 (100 %, MH^+) and 299 (27 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 299.1256, $C_{16}H_{20}NaO_4$ requires *MNa* 299.1259. **IR** ν_{max}(liquid film): 2939 (CH), 1705 (C=O) and 1601 (C=C). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.8.

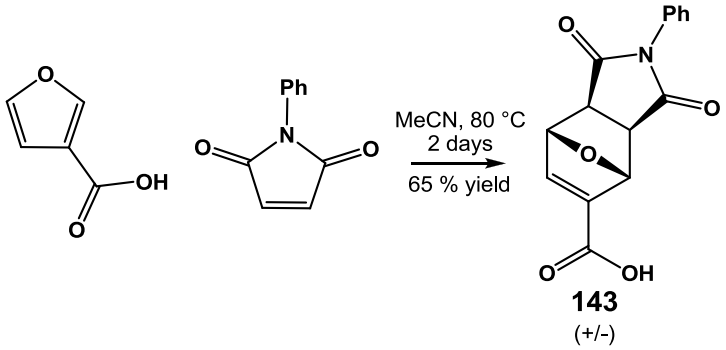
Synthesis of (3R,4S,5R)-3,4,5-triacetoxycyclohex-1-enecarboxylic acid 140



139 **140**

Following a procedure reported by Streicher *et al.*,[123] shikimic acid **139** (174 mg, 1 mmol, 1 eq.) dissolved in pyridine (2 mL) and acetic anhydride (1 mL) was stirred at room temperature for 5 hours. The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (10 mL), washed with 1M $HCl_{(aq.)}$ (2 x 10 mL), brine (10 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], compound **140** (298 mg, 99 % yield) was isolated as a colourless oil (Srinavas *et al.*[188] report this compound as white crystals). **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 6.82 (1H, t, *J* = 2.0 Hz, alkene C*H*), 5.76-5.74 (1H, m, OC*H*), 5.31-5.25 (2H, m, OC*H*), 2.91-2.85 (1H, m, C*Ha*Hb), 2.45-2.40 (1H, m, CHa*Hb*), 2.08 (3H, s, CH_3), 2.07 (3H, s, CH_3) and 2.05 (3H, s, CH_3). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 170.3- ((*C*=O)OH), 170.0- and 169.9- (3 x (*C*=O)), 135.1+ (alkene *C*H), 130.5- (alkene*C*), 67.4+ (O*C*H), 66.7+ (O*C*H), 66.0+ (O*C*H), 28.0- (*C*H_2), 21.0+ and 20.7+ (3 x *C*H_3). **IR** ν_{max}(liquid film): 1728 (C=O) and 1655 (C=O). **MS** *m/z* (+ESI) 323 (100 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 323.0784, $C_{13}H_{16}NaO_8$ requires *MNa* 323.0743 (13 ppm). **Rf** (100 % EtOAc) 0.5. Spectroscopic data are consistent with those reported by Streicher *et al.*[123]

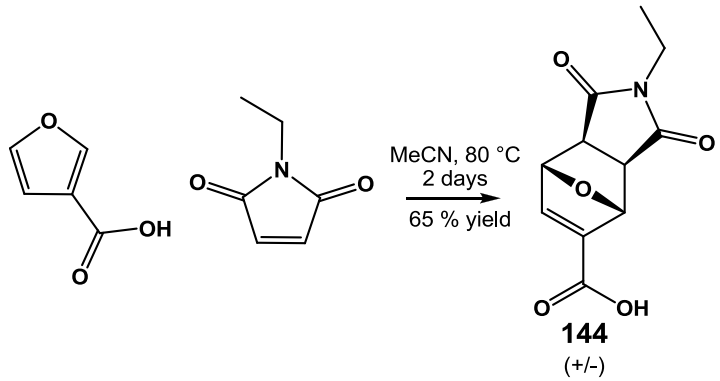
Synthesis of Diels-Alder product 143



143
(+/-)

N-Phenylmaleimide (520 mg, 3 mmol, 1 eq.) and 3-furoic acid (672 mg, 6 mmol, 2 eq.) were dissolved in MeCN (20 mL) and heated at 80 ^{o}C for 2 days. The reaction mixture was cooled and the solvent was removed under reduced pressure. Et_2O was added and the white solid was filtered and dried to collect acid **143** (560 mg, 65 % yield) as a white solid, **m.p.** >220 ^{o}C. **^{1}H NMR** (400 MHz; $(CD_3)_2SO$): δ_H 7.58-7.47 (3H, m, ArCH), 7.33 (1H, d, *J* = 1.5 Hz, alkene C*H*), 7.29-7.23 (2H, m, ArCH), 5.47 (1H, s, (CO)*H*), 5.40 (1H, s, (CO)*H*), 3.33 (1H, d, *J* = 6.5 Hz, (C=O)C*H*) and 3.23 (1H, d, *J* = 6.5 Hz, (C=O)C*H*). **^{13}C NMR** (100 MHz; $(CD_3)_2SO$): δ_C 175.1- (*C*=O)N), 175.0- (*C*=O)N), 163.3- ((*C*=O)OH), 145.1+ (alkene *C*H), 142.7- (*C*), 132.0- (*C*), 129.0+ (Ar*C*H), 128.5+ (Ar*C*H), 126.8+ (Ar*C*H), 82.3+ (*C*-O), 80.4+ (*C*-O), 47.4+ ((C=O)*C*H) and 47.3+ ((C=O)*C*H). **MS** *m/z* (ESI+) 308 (19 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 308.0562, $C_{15}H_{11}NNaO_5$ requires *MNa* 308.0535 (9 ppm). **IR** ν_{max}(liquid film): 2990 (CH), 1783 (C=O), 1718 (C=O) and 1599 (C=C).

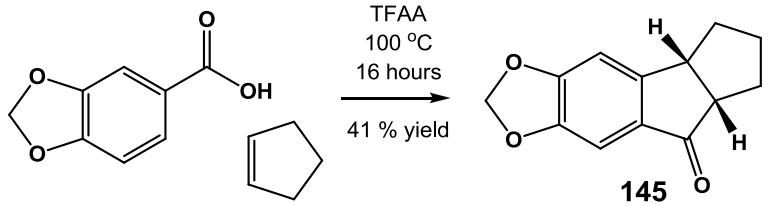
Synthesis of Diels-Alder product 144



144
(+/-)

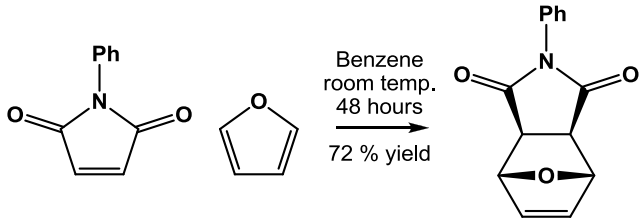
N-Ethylmaleimide (375 mg, 3 mmol, 1 eq.) and 3-furoic acid (672 mg, 6 mmol, 2 eq.) were dissolved in MeCN (20 mL) and heated at 80 ^{o}C for 2 days. The reaction mixture was cooled and the solvent was removed under reduced pressure. Et_2O was added and the white solid was filtered and dried to collect acid **144** (465 mg, 65 % yield) as a white solid, **m.p.** >220 ^{o}C. **^{1}H NMR** (400 MHz; $(CD_3)_2SO$): δ_H 7.20 (1H, d, *J* = 2.0 Hz, alkene C*H*), 5.27 (1H, d, *J* = 1.5 Hz, (CO)*H*), 5.19 (1H, s, (CO)*H*), 3.38 (2H, q, *J* = 7.0 Hz, CH_2), 3.07 (1H, d, *J* = 7.0 Hz, C*H*(C=O)), 2.98 (1H, d, *J* = 7.0 Hz, C*H*(C=O)) and 1.00 (3H, t, *J* = 7.0 Hz, CH_3). **^{13}C NMR** (100 MHz; $(CD_3)_2SO$): δ_C 175.6- (*C*=O)N), 175.6- (*C*=O)N), 163.3- ((*C*=O)OH), 145.0+ (alkene *C*H), 142.5- (*C*), 81.4+ *C*HO), 79.9+ (*C*HO), 47.1+ (*C*H(C=O)), 47.0+ (*C*H(C=O)) 33.1- (*C*H_2) and 12.7+ (*C*H_3). **MS** *m/z* (ESI+) 260 (63 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 260.0546, $C_{11}H_{11}NNaO_5$ requires *MNa* 260.0535. **IR** ν_{max}(liquid film): 1783 (C=N), 1705 (C=O) and 1604 (C=C).

Synthesis of indanone 145



Following **General Procedure 10**, piperonylic acid on a 2 mmol scale and cyclopentene (2 eq.) in TFAA (3.5 eq.) at 100 ^{o}C for 16 hours, afforded the indanone **145** (178 mg, 41 % yield) as a yellow solid, **m.p.** 80-83 ^{o}C. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.01 (1H, s, ArC*H*C(C=O)), 6.82 (1H, s, ArC*H*CCH), 6.05-6.04 (2H, m, OCH_2O), 3.64-3.60 (1H, m, (CO)C*H*CH), 3.04 (1H, ddd, *J* = 10.0, 7.0, 2.0 Hz, (CO)CHC*H*), 2.04-1.74 (4H, m, CH_2CH_2), 1.60-1.56 (1H, m, CH_2) and 1.19-1.13 (1H, m, CH_2). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 207.8- (*C*=O), 156.2- (*C*), 154.5- (*C*), 148.3- (*C*), 132.1- (*C*) 104.9+ (Ar*C*HCCH), 102.1+ (Ar*C*HC(C=O)), 101.6- (O*C*H_2O), 52.9+ ((CO)CH*C*H), 43.7+ ((CO)*C*HCH), 32.8- (*C*H_2), 30.6- (*C*H_2) and 24.4- (*C*H_2). **MS** *m/z* (+ESI) 217 (100 %, MH^+) and 239 (70 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 239.0702, $C_{13}H_{13}NNaO_2$ requires *MNa* 239.0684 (8 ppm). **IR** ν_{max}(liquid film): 2958 (CH), 1693 (C=O) and 1609 (C=C). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.7.

Synthesis of Diels-Alder product 146

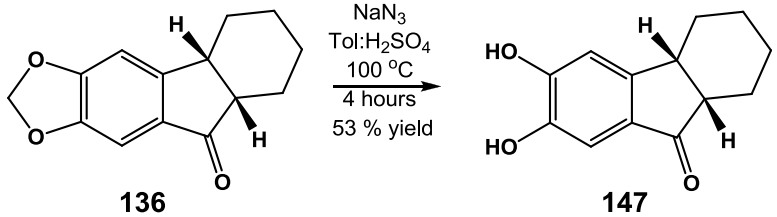


146

N-Phenylmaleimide (693 mg, 4 mmol, 1 eq.) and furan (0.58 mL, 8 mmol, 2 eq.) in THF (6 mL) were stirred at room temperature for 48 hours. The reaction mixture was concentrated under reduced pressure to collect 884 mg of yellow solid. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], compound **146** (694 mg, 72 % yield) was collected as a white solid, **m.p.** 150-152 ^{o}C [lit.[189] 164-165 ^{o}C]. **^{1}H NMR** (400 MHz, $CDCl_3$): δ_H 7.48-7.43 (2H, m, ArC*H*), 7.38 (1H, tt, *J* = 8.0, 1.0 Hz, ArC(4)*H*), 7.28-7.26 (2H, m, ArC*H*), 6.55 (2H, t, *J* = 1.0 Hz, alkene C*H*), 5.38 (2H, t, *J* = 1.0 Hz, (CO)*H*) and 2.99 (2H, s, C*H*(C=O)). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ_C 175.3- (*C*=O)), 136.7+ (alkene *C*H), 131.8- (Ar*C*(1)), 129.1- (Ar*C*H), 128.8+ (Ar*C*H), 126.6+ (Ar*C*H), 81.5+ (*C*O)H), 47.6+ (*C*H(C=O). **MS** *m/z* (+ESI) 264 (91 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 264.0623, $C_{14}H_{11}NNaO_3$ requires *MNa* 264.0637. **IR** ν_{max}(liquid film): 2988 (CH), 1714 (C=O) and 1598 (C=C). **Rf** (40 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.4. Spectroscopic data are consistent with those reported by Riande *et al.*[189]

7.5.2. Schmidt Reaction and Beckmann Rearrangement

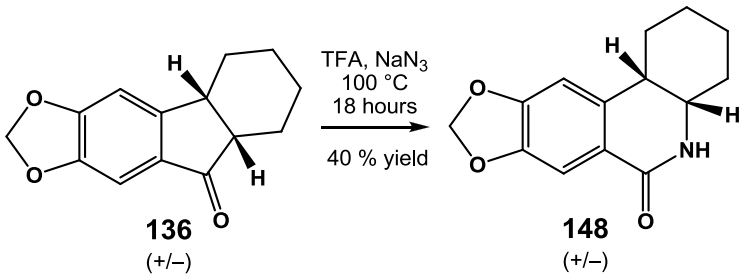
Synthesis of (4aR,9aS)-6,7-dihydroxy-2,3,4,4a-tetrahydro-1H-fluoren-9(9aH)-one 147



136 (+/–) **147** (+/–)

NaN_3 (338 mg, 5.20 mmol, 4 eq.) was added to **136** (300 mg, 1.30 mmol, 1 eq.) in toluene: H_2SO_4 (2.5 mL: 2.5 mL). The reaction mixture was heated at 100 ^oC for 4 hours; on cooling, sat. aq. $NaHCO_3$ (15 mL) and EtOAc (15 mL) were added and the mixture was stirred at room temperature for 10 minutes. The aqueous layer was extracted with EtOAc (4 x 15 mL); the combined organic layers were washed with brine (15 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure. The aqueous layer was extracted with $CHCl_3$ (4 x 15 mL), the combined organic layers were washed with brine (15 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure. Both the crude products were combined and, after column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], indanone **147** (151 mg, 53 % yield) was isolated as a white solid, **m.p.** 199-202 ^oC. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.21 (1H, s, ArC*H*C(C=O)), 6.86 (1H, s, ArC*H*CCH), 3.20 (1H, dt, *J* = 15.0, 7.0 Hz, (CO)C*H*CH), 2.65 (1H, dt, *J* = 13.0, 7.0 Hz, (CO)CHC*H*) and 2.09-1.16 (8H, m, (CH_2)$_4$). **MS** *m/z* (+ESI) 219 (100 %, MH^+) and 241 (71 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 241.0843, $C_{13}H_{14}NaO_3$ requires *MNa* 241.0841. **IR** ν_{max}(liquid film): 3600 (OH) and 1701 (C=O). **Rf** (40 % EtOAc in light petroleum (b.p. 40-60 ^oC)) 0.3.

Synthesis of 1,2,3,4,4a,5-hexahydro-[1,3]dioxolo[4,5-j]phenanthridin-6(11bH)-one 148



Method A

Following the procedure reported by Irie *et al.*,[98] indanone **136** (248 mg, 1.08 mmol, 1 eq.) was dissolved in trichloroacetic acid (3 mL) and the reaction mixture was heated to 60 ^{o}C. NaN_3 (280 mg, 4.32 mmol, 4 eq.) was added slowly portionwise over 6 hours (1.08 mmol every 2 hours). The reaction mixture was left to stir for a further two days and the reaction mixture was neutralised with 1M $NaOH_{(aq.)}$. The aqueous layer was extracted with $CHCl_3$ (3 x 30 mL) and the combined organic layers were washed with brine, dried on Na_2SO_4 and filtered and the solvent was removed under reduced pressure to afford a crude orange oil. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the lactam **148** (50 mg, 19 % yield) was isolated as a yellow solid.

Method B

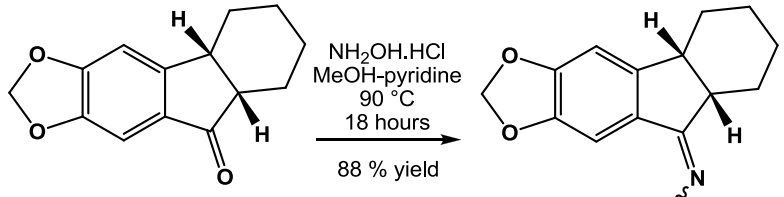
Following the procedure reported by Jesudason *et al.*,[96] NaN_3 (249 mg, 3.84 mmol, 4 eq.) was added to indanone **136** (220 mg, 0.96 mmol, 1 eq.) in toluene: H_2SO_4 (0.5 mL: 0.5 mL) and the reaction mixture was stirred at room temperature for 4 days. Sat. aq. $NaHCO_3$ (20 mL) and EtOAc (20 mL) were added and the reaction mixture stirred at room temperature for 1 hour. The aqueous layer was extracted with EtOAc (2 x 20 mL), the combined organic layers were washed with brine (20 mL), dried on Na_2SO_4 and filtered and the solvent was removed under reduced pressure to afford a crude brown solid. The aqueous layer was re-extracted with $CHCl_3$ (3 x 20 mL) and the combined organic layers were washed with brine (20 mL), dried on Na_2SO_4 and filtered and the solvent was removed under reduced pressure to afford a second portion of crude brown solid. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], the lactam **148** (50 mg, 21 % yield) was isolated as a yellow solid.

Method C - Optimised Method

Following **General Procedure 7**, using indanone **136** (95 mg, 0.41 mmol, 1 eq.) and NaN_3 (107 mg, 1.64 mmol, 4 eq.) in TFA (1 mL), the lactam **148** (40 mg, 40% yield) was isolated as a yellow solid.

m.p. 259-261 ^{o}C. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.44 (1H, s, ArC*H*C(C=O)), 6.57 (1H, s, ArC*H*CCH), 5.93 (2H, m, OCH_2O), 5.54 (1H, br.s. N*H*), 3.81 (1H, m, NC*H*CH), 2.61 (1H, m, NCHC*H*) and 1.74-1.25 (8H, m, (CH_2)$_4$). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 166.3- (*C*=O), 151.0- (Ar*C*-O), 146.8- (Ar*C*-O), 137.1- (Ar*C*), 121.5- (Ar*C*), 108.0+ (Ar*C*HC(C=O)), 106.7+ (Ar*C*HCCH), 101.5- (O*C*H_2O), 50.1+ (N*C*HCH), 40.3+ (NCH*C*H), 30.1- (*C*H_2), 29.7- (*C*H_2), 29.2- (*C*H_2) and 19.8- (*C*H_2). **MS** *m/z* (ESI+) 246 (39 %, MH^+) and 268 (8.4 %, MNa^+). **HRMS** (+ESI) Found MH^+ 246.1118, $C_{14}H_{15}NO_3$ requires *MH* 246.1130 and found MNa^+ 268.0939, $C_{14}H_{14}NNaO_3$ requires *MNa* 268.0949. **IR** ν_{max}(liquid film): 3402 (NH), 2987 (CH) and 1664 (C=O). **Rf** (100 % EtOAc) 0.5.

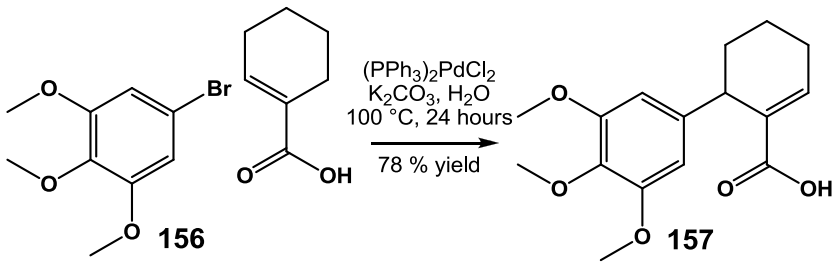
Synthesis of 6,7,8,8a-tetrahydro-4bH-fluoreno[2,3-d][1,3]dioxol-9(5H)-one oxime 150



$NH_2OH.HCl$ (129 mg, 1.85 mmol, 2.5 eq.) was added to a solution of indanone **136** (171 mg, 0.74 mmol, 1 eq.) dissolved in MeOH-pyridine (2 mL: 2 mL) and the reaction mixture was heated at 90 ^oC for 18 hours. The reaction mixture was cooled and concentrated under reduced pressure to collect oxime **150** (159 mg, 88 % yield) as a white solid, **m.p.** 195-197 ^oC. **^{1}H NMR** (400 MHz; $(CD_3)_2SO$): δ_H 10.80 (1H, br, O*H*), 7.74 (1H, s, ArC*H*C(C=N)), 7.01 (1H, s, ArC*H*CCH), 6.10 (2H, d, *J* = 2.5 Hz, OCH_2O), 3.12 (1H, q, *J* = 7.0 Hz, (C=N)C*H*CH), 2.98 (1H, q, *J* = 6.5 Hz, (C=N)CHC*H*) and 1.93-1.64 (8H, m, $(CH_2)_4$). **^{13}C NMR** (100 MHz; $(CD_3)_2SO$): δ_C 157.7- (*C*=N), 149.1- (*C*), 147.1- (*C*), 145.9- (*C*), 126.2- (*C*), 108.3+ (Ar*C*HC(C=N)), 104.5+ (Ar*C*HCCH), 101.1- (O*C*H_2O), 42.2- (C=N)CH*C*H), 40.3- (C=N)*C*HCH), 29.5- (*C*H_2), 25.7- (*C*H_2), 22.0- (*C*H_2) and 21.9 (*C*H_2). **MS** *m/z* (+ESI) 246 (100 %, MH^+) and 268 (13 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 268.0935, $C_{14}H_{15}NNaO_3$ requires *MNa* 268.0950. **IR** ν_{max}(liquid film): 3600 (OH), 2933 (CH) and 1470 (C=N).

7.5.3. Heck Reaction

Synthesis of 6-(3,4,5-trimethoxyphenyl)cyclohex-1-ene carboxylic acid 157



Method A

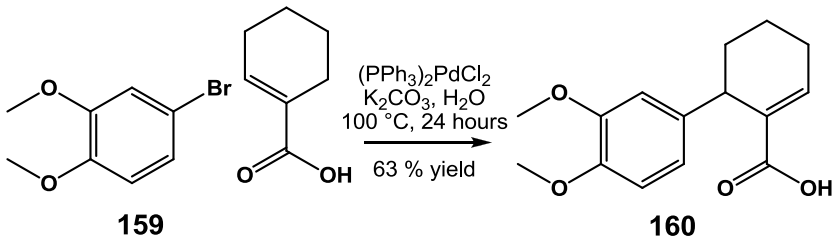
Following a procedure reported by Davidson *et al.*[133] but using K_2CO_3 as a base and $(PPh_3)_2PdCl_2$ as the Pd source, 3,4,5-trimethoxy-5-bromobenzene **156** (494 mg, 2 mmol, 2 eq.), cyclohexene-1-carboxylic acid (126 mg, 1 mmol, 1 eq.) and K_2CO_3 (415 mg, 3 mmol, 3 eq.) in H_2O (8 mL) were degassed and placed under an Ar atmosphere. $(PPh_3)_2PdCl_2$ (70 mg, 0.1 mmol, 10 mol %) was then added, the reaction mixture was degassed for a second time and placed under an Ar atmosphere. The reaction mixture was stirred at 100 ^oC for 24 hours; on cooling, the reaction mixture was filtered through a pad of celite and the celite was washed with sat. aq. $NaHCO_3$. The aqueous layer was washed with Et_2O (3 x 20 mL), which was discarded and the aqueous layer was acidified using 6M $HCl_{(aq.)}$. This was then extracted with EtOAc (3 x 20 mL), the combined organic layers were washed with brine (20 mL), dried on $NaSO_4$ and filtered and the solvent was removed under reduced pressure to collect 197 mg of crude brown oil. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], **157** (127 mg, 43 % yield) was isolated as a white solid.

Method B - Optimised Method

Following a procedure reported by Davidson *et al.*[133] but using K_2CO_3 as a base and $(PPh_3)_2PdCl_2$ as the Pd source, 3,4,5-trimethoxy-5-bromobenzene **156** (494 mg, 2 mmol, 2 eq.), cyclohexene-1-carboxylic acid (126 mg, 1 mmol, 1 eq.) and K_2CO_3 (415 mg, 3 mmol, 3 eq.) in H_2O (8 mL) were degassed and placed under an Ar atmosphere. $(PPh_3)_2PdCl_2$ (70 mg, 0.1 mmol, 10 mol %) was then added, the reaction mixture was degassed for a second time and placed under an Ar atmosphere. The reaction mixture was stirred at 100 ^oC for 24 hours, on cooling the reaction mixture was acidified using 6M $HCl_{(aq.)}$ and the water was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column] **157** (229 mg, **78 % yield**) was isolated as a white solid, **m.p.** 103-

105 ^{o}C. **^{1}H NMR** (400MHz, $CDCl_3$): δ_H 7.36 (1H, t, *J* = 3.5 Hz, C*H*-alkene), 6.31 (2H, s, ArC(2)*H*), 3.84 (1H, m, ArC(1)C*H*), 3.81 (6H, s, OCH_3), 3.80 (3H, s, OCH_3), 2.34-2.26 (2H, m, CH_2), 1.86-1.75 (2H, m, CH_2) and 1.53-1.49 (2H, m, CH_2). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 172.2- (*C*=O), 152.9- (Ar*C*(3)), 144.5+ (*C*H-alkene), 140.4- (Ar*C*(4)), 136.1- (Ar*C*(1)), 131.1- (*C*(C=O)), 104.7+ (Ar*C*(2)H), 60.7+ (O*C*H_3), 56.0+ (O*C*H_3), 39.1+ (ArC(1)*C*H), 31.2- (*C*H_2), 25.0- (*C*H_2) and 16.7- (*C*H_2). **MS** *m/z* (+ESI) 293 (100 %, MH^+) and 315 (25 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 315.1213, $C_{16}H_{20}NaO_5$ requires *MNa* 315.1208.

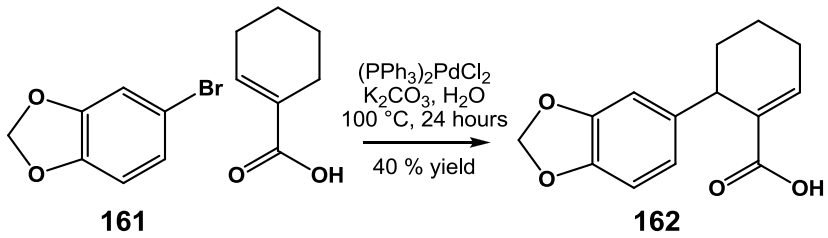
Synthesis of 6-(3,4-dimethoxyphenyl)cyclohexene-1-carboxylic acid 160



159 **160**

4-Bromoveratrole **159** (0.29 mL, 2 mmol, 1 eq.), cyclohexene-1-carboxylic acid (126 mg, 1 mmol, 1 eq.) and K_2CO_3 (415 mg, 3 mmol, 3 eq.) in H_2O (8 mL) were degassed and placed under an Ar atmosphere. $(PPh_3)_2PdCl_2$ (70 mg, 0.1 mmol, 10 mol %) was then added, the reaction mixture was degassed for a second time and placed under an Ar atmosphere. The reaction mixture was stirred at 100 ^oC for 24 hours, on cooling the reaction mixture was acidified using 6M $HCl_{(aq.)}$ and the water removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], **160** (165 mg, 63 % yield) was isolated as a white solid, **m.p.** 108-111 ^oC. **^{1}H NMR** (400MHz, $CDCl_3$): δ_H 7.33 (1H, t, *J* = 4.0 Hz, C*H*-alkene), 6.74 (1H, d, *J* = 8.0 Hz, ArC(5)*H*), 6.67 (1H, d, *J* = 2.0 Hz, ArC(2)*H*), 6.61 (1H, dd, *J* = 8.0, 2.0 Hz, ArC(6)*H*), 3.84-3.82 (1H, m, ArC(1)C*H*), 3.84 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 2.38-2.18 (2H, m, CH_2), 1.90-1.72 (2H, m, CH_2) and 1.53-1.46 (2H, m, CH_2). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 172.0- (*C*=O), 148.8- (Ar*C*(3)), 147.3- (Ar*C*(4)), 143.9+ (*C*H-alkene), 137.3- (Ar*C*(1)), 131.3- (*C*(C=O)), 119.5+ (Ar*C*(6)H), 111.3+ (Ar*C*(2)H), 110.8+ (Ar*C*(5)H), 55.8+ (O*C*H_3), 55.8+ (O*C*H_3), 38.5+ (ArC(1)*C*H), 31.2- (*C*H_2), 26.0- (*C*H_2) and 16.6- (*C*H_2). **MS** *m/z* (+ESI) 263 (18 %, MH^+). **HRMS** (+ESI) Found MH^+ 263.1279, $C_{15}H_{19}O_4$ requires *MH* 263.1283. **IR** ν_{max}(liquid film): 2950 (OH), 1687 (C=O) and 1515 (C=C).

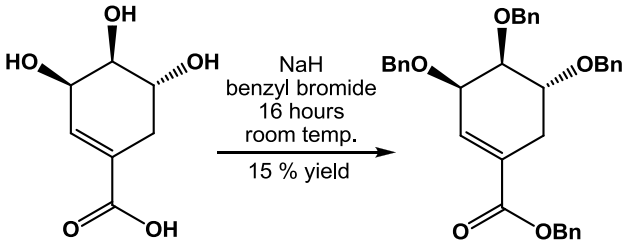
Synthesis of 6-(benzo[*d*][1,3]dioxol-5-yl)cyclohexene-1-carboxylic acid 162



161 **162**

5-Bromo-1,3-benzodioxole **161** (0.24 mL, 2 mmol, 1 eq.), cyclohexene-1-carboxylic acid (126 mg, 1 mmol, 1 eq.) and K_2CO_3 (415 mg, 3 mmol, 3 eq.) in H_2O (8 mL) were degassed and placed under an Ar atmosphere. $(PPh_3)_2PdCl_2$ (70 mg, 0.1 mmol, 10 mol%) was then added, the reaction mixture was degassed for a second time and placed under an Ar atmosphere. The reaction mixture was stirred at 100 ^oC for 24 hours; on cooling, the reaction mixture was acidified using 6M $HCl_{(aq.)}$ and the water was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], **162** (99 mg, 40 % yield) was isolated as a white solid, **m.p.** 163-166 ^oC. **^{1}H NMR** (400MHz, $CDCl_3$): δ_H 7.33 (1H, t, *J* = 3.5 Hz, C*H*-alkene), 6.70 (1H, d, *J* = 8.0 Hz, ArC(5)*H*), 6.62 (1H, d, *J* = 2.0 Hz, (ArC(2)*H*), 6.56 (1H, dd, *J* = 8.0, 2.0 Hz, ArC(6)*H*), 5.90 (2H, s, OCH_2O), 3.82 (1H, br.s. ArC(1)C*H*), 2.37-2.17 (2H, m, CH_2) and 1.90-1.46 (4H, m, CH_2CH_2). **^{13}C NMR** (100 MHz, $CDCl_3$): δ_C 171.8- (*C*=O), 147.4- (Ar*C*(3)), 145.8- (Ar*C*(4)), 144.1+ (*C*H-alkene), 138.6- (Ar*C*(1)), 131.3- (*C*(C=O)), 120.6+ (Ar*C*(6)H), 108.3+ (Ar*C*(2)H), 107.9+ (Ar*C*(5)H), 100.7- (O*C*H_2O), 38.6+ (ArC(1)*C*H), 31.2- (*C*H_2), 26.0- (*C*H_2) and 16.4- (*C*H_2). **MS** *m/z* (+ESI) 247 (20 %, MH^+) and 269 (100 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 269.0791, $C_{14}H_{14}NaO_4$ requires *MNa* 269.0790.

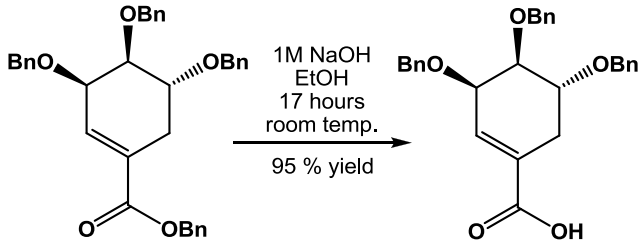
Synthesis of benzyl-(3R,4S,5R)-3,4,5-tri(benzyloxy)cyclohexene-1-carboxylate 163



139 **163**

Following a procedure reported by Evers *et al*.,[143] shikimic acid **139** (174 mg, 1 mmol, 1 eq.) was dissolved in DMF (3 mL) and cooled to 0 ^{o}C. NaH (60% in mineral oil) (180 mg, 4.5 mmol, 4.5 eq.) was added and the mixture was stirred for 30 minutes. Benzyl bromide (0.48 mL, 4.05 mmol, 4.05 eq.) was added and the reaction mixture stirred at room temperature for 16 hours. The solvent was removed under reduced pressure, the residue was dissolved in H_2O (10 mL), extracted with Et_2O (10 mL), acidified with 5 % aqueous citric acid and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], compound **163** (78 mg, 15 % yield) was isolated as a colourless oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.38-7.22 (20H, m, ArC*H*), 6.97 (1H, s, alkene C*H*), 5.18 (2H, m, PhC*H*$_2$), 4.75-4.66 (2H, m PhC*H*$_2$), 4.66 (2H, s, PhC*H*$_2$), 4.60-4.49 (2H, m, PhC*H*$_2$), 4.35 (1H, s, OC*H*), 3.98-3.95 (1H, m, OC*H*), 3.85-3.82 (1H, m, OC*H*), 2.77-2.71 (1H, m, C*Ha*Hb) and 2.51-2.45 (1H, m, CHa*Hb*). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 166.3- (*C*=O), 138.4- (*C*), 138.2- (*C*), 136.7- (*C*), 135.9- (*C*), 130.9- (*C*), 129.1+ (*C*H), 128.8+ (*C*H), 128.5+ (*C*H), 128.4+ (*C*H), 128.3+ (*C*H), 128.2+ (*C*H), 128.1+ (*C*H), 128.0+ (*C*H), 127.8+ (*C*H), 127.7+ (*C*H), 127.7+ (*C*H), 127.7+ (*C*H), 127.5+ (*C*H), 74.7+ (O*C*H), 73.6+ (O*C*H), 73.2+ (O*C*H), 72.9- (*C*H$_2$), 71.7- (*C*H$_2$), 71.6- (*C*H$_2$), 66.4- (*C*H$_2$) and 60.4- (*C*H$_2$). **IR** ν_{max}(liquid film): 2999 (CH) and 1713 (C=O). **MS** *m/z* (+ESI) 557 (12 %, MNa^{+}). **HRMS** (+ESI) Found 557.2284, $C_{35}H_{34}NaO_5$ requires *MNa* 557.2304. **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.8.

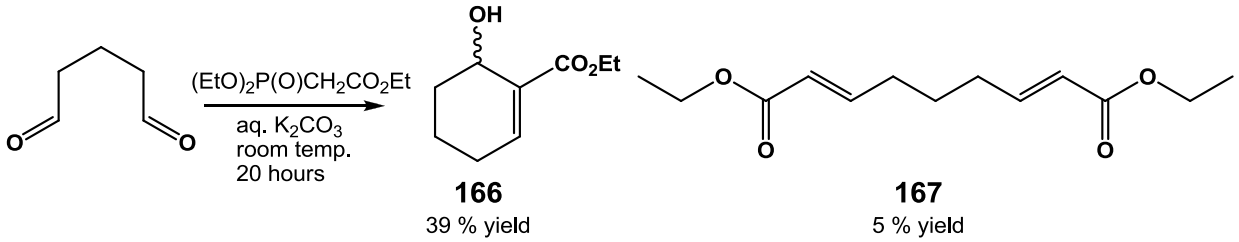
Synthesis of (3R,4S,5R)-3,4,5-tri(benzyloxy)cyclohex-1-ene carboxylic acid 164



163 164

Ester **163** (217 mg, 0.42 mmol, 1 eq.) in EtOH (1.5 mL) was added to 1M $NaOH_{(aq.)}$ (0.5 mL, 0.50 mmol, 1.2 eq.). The mixture was stirred at room temperature for 1 hour, upon which another aliquot of 1M $NaOH_{(aq.)}$ (0.5 mL, 0.5 mmol, 1.2 eq.) was added. Stirring was continued for 16 hours, the reaction mixture was diluted with H_2O (10 mL) and washed with PE (3 x 10 mL) which was then discarded. The aqueous layer was acidified with $HCl_{(aq.)}$ and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (10 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure to afford **164** (172 mg, 95 % yield) as a colouress oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.38-7.22 (15H, m, ArC*H*), 7.06 (1H, s, alkene C*H*), 4.75-4.65 (4H, m, PhCH_2), 4.58 (1H, d, *J* = 10.0 Hz, PhC*Ha*Hb), 4.50 (1H, d, *J* = 10.0 Hz, PhCHa*Hb*), 4.37 (1H, s, OC*H*), 3.98-3.95 (1H, m, OC*H*), 3.86-3.84 (1H, m, OC*H*), 2.72-2.68 (1H, m, C*Ha*Hb) and 2.47-2.43 (1H, m, CHa*Hb*). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 171.4- (*C*=O), 138.9- (*C*H), 138.4- (*C*), 138.1- (*C*), 128.4- (*C*), 128.4- (*C*H), 128.3+ (*C*H), 127.8+ (*C*H), 127.8+ (*C*H), 127.7+ (*C*H), 127.7+ (*C*H), 127.7+ (*C*H), 127.5+ (*C*H), 127.3+ (*C*H), 74.8+ (O*C*H), 73.6+ (O*C*H), 73.2+ (O*C*H), 73.0- (CH_2), 71.7- (CH_2), 71.5- (CH_2) and 27.3- (CH_2). **IR** ν_{max}(liquid film): 2990 (CH) and 1694 (C=O). **MS** *m/z* (+ESI) 467 (81 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 467.1865, $C_{28}H_{28}NaO_5$ requires *MNa* 467.1834 (6.6 ppm).

Synthesis of ethyl 6-hydroxycyclohex-1-ene carboxylate 166 and (2E,7E)-diethyl nona-2,7-dienedioate 167



166 39 % yield **167** 5 % yield

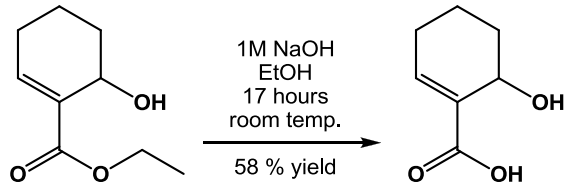
Following a procedure reported by Iwabuchi *et al.*[145] but with triethyl phosphonoacetate, 6.4M $K_2CO_{3(aq.)}$(1.95 mL, 12.5 mmol, 1 eq.) was added to 25 % aqueous glutaraldehyde (2 mL, 5 mmol, 1 eq.) in triethyl phosphonoacetate (0.99 mL, 5 mmol, 1 eq.). The reaction mixture was left to stir at room temperature for 20 hours. The reaction mixture was diluted with H_2O (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried on Na_2SO_4 and filtered and the solvent was removed under reduced pressure to collect 1.26 g of colourless oil. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], compounds **166** (335 mg, 39 % yield) and **167** (62 mg, 5 % yield) were isolated as colourless oils.

Ester 166 ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 7.05 (1H, t, *J* = 4.0 Hz, alkene C*H*), 4.51-4.49 (1H, m, C*H*OH), 4.19 (2H, q, *J* = 7.0 Hz, OC*H*$_2$CH$_3$), 3.14 (1H, br.s. O*H*), 2.27-2.06 (2H, m, C*H*$_2$), 1.83-1.54 (4H, m, C*H*$_2$C*H*$_2$) and 1.27 (3H, t, *J* = 7.0 Hz, OCH$_2$C*H*$_3$). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 167.3- (*C*=O), 142.7+ (alkene *C*H), 122.2- (*C*), 63.4+ (*C*HOH), 60.5- (O*C*H$_2$CH$_3$), 29.8- (*C*H$_2$), 26.0- (*C*H$_2$), 17.5- (*C*H$_2$) and 14.1+ (OCH$_2$*C*H$_3$). **HRMS** (+ESI) Found MH^+ 171.1029, $C_9H_{15}O_3$ requires *MH* 171.1021. **IR** ν_{max}(liquid film): 3600 (OH), 2949 (CH) and 1701 (C=O). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^oC)) 0.4. Spectroscopic data are consistent with those reported by Alexakis *et al.*[190]

Diester 167 ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 6.90 (2H, dt, *J* = 15.5, 7.0 Hz, CO$_2$CHC*H*CH$_2$), 5.80 (2H, dt, *J* = 15.5, 1.5 Hz, CO$_2$C*H*CHCH$_2$), 4.15 (4H, q, *J* = 7.0 Hz, CH$_3$C*H*$_2$CO$_2$), 2.20 (4H, qd, *J* = 7.5, 1.5 Hz, CO$_2$CHCHC*H*$_2$), 1.61 (2H, quint. *J* = 7.5 Hz, CH$_2$) and 1.26 (6H, t, *J* = 7.0 Hz, C*H*$_3$CH$_2$CO$_2$). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 166.4- (*C*=O), 147.9+ (CO$_2$CH*C*HCH$_2$), 121.9+ (CO$_2$*C*HCHCH$_2$), 60.1- (CH$_3$*C*H$_2$CO$_2$), 31.3- (CO$_2$CHCH*C*H$_2$), 26.3- (*C*H$_2$) and 14.2+ (*C*H$_3$CH$_2$CO$_2$). **MS** *m/z* (+ESI) 241 (12 %, MH^+) and 263 (100 %, MNa^+). **HRMS** (+ESI) Found MH^+ 241.1448, $C_{13}H_{21}O_4$ requires *MH* 241.1440. **IR**

ν_{max}(liquid film): 2975 (CH) and 1718 (C=O). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C)) 0.8.

Synthesis of 6-hydroxycyclohex-1-ene carboxylic acid 168

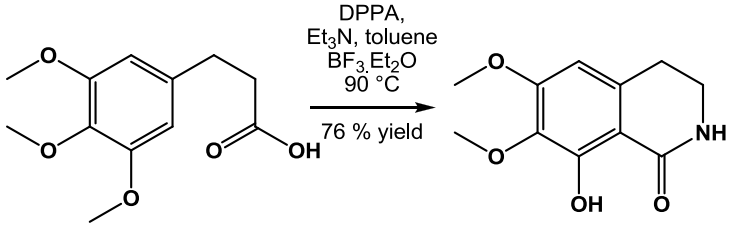


166 **168**

Following a procedure reported by Bultman *et al.*,[146] ester **166** (267 mg, 1.57 mmol, 1 eq.) in EtOH (2 mL) was added to 1M $NaOH_{(aq.)}$ (1.88 mL, 1.88 mmol, 1.2 eq.). The mixture was stirred at room temperature for 1 hour, upon which another aliquot of 1M $NaOH_{(aq.)}$ (1.88 mL, 1.88 mmol, 1.2 eq.) was added. Stirring was continued for 16 hours and the reaction mixture was diluted with H_2O (20 mL) and washed with PE (3 x 10 mL) which were then discarded. The aqueous layer was acidified with $HCl_{(aq.)}$ and was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (10 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure to afford **168** (128 mg, 58 % yield) as a colourless oil. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.25 (1H, t, *J* = 4.0 Hz, alkene C*H*), 4.55 (1H, t, *J* = 4.0 Hz, C*H*OH), 2.35-2.14 (2H, m, CH_2) and 1.90-1.77 (4H, m, CH_2CH_2). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 171.7- (*C*=O), 146.0+ (alkene *C*H), 131.5- (*C*), 63.2+ (*C*HOH), 29.8- (*C*H_2), 26.3- (*C*H_2) and 17.2- (*C*H_2). **MS** *m/z* (+ESI) 165 (100 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 165.0533, $C_7H_{10}NaO_3$ requires *MNa* 165.0522 (6.6 ppm). **IR** ν_{max}(liquid film): 3600 (OH), 2951 (OH) and 1696 (C=O).

7.5.4. Modified Curtius Rearrangement

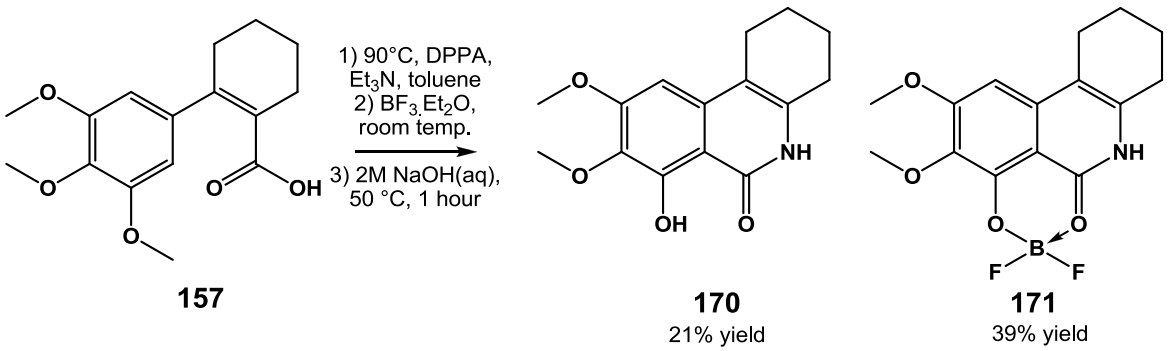
Synthesis of 8-hydroxy-6,7-dimethoxy-3,4-dihydroisoquinolin-1(2H)-one 126



126

Following a procedure reported by Judd *et al.*,[93] diphenylphosphoryl azide (0.65 mL, 3 mmol, 1 eq.) was added dropwise to a stirred solution of 3-(3,4,5-trimethoxyphenyl)propanoic acid (721 mg, 3 mmol, 1 eq.) and Et_3N (0.42 mL, 3 mmol, 1 eq.), in anydrous toluene (10 mL) at room temperature under N_2. The reaction mixture was then heated with stirring at 90^{o}C for 90 minutes. Most of the solvent was removed under reduced pressure to afford a mobile oil and the flask was cooled to 0^{o}C under N_2. $BF_3.Et_2O$ (2 mL) was added dropwise to the rapidly stirred reaction mixture. The reaction mixture was warmed to room temperature and left to stir for 16 hours. The reaction mixture was quenched with aq. 1M NaOH (to pH 10) and EtOAc (10 mL) added. The rapidly stirred mixture was heated to 50^{o}C for 1 hour, dissolving all the crude material. The mixture was cooled to room temperature, the layers separated and the aqueous layer was further extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried on $NaSO_4$ and filtered and the solvent was removed to give 1g of the crude product. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], **126** (511 mg, 76 % yield) was isolated as a white solid, **m.p.** 179-182 ^{o}C, [lit.[93] 181-183 ^{o}C]. **^{1}H NMR** (400 MHz, $CDCl_3$): δ_H 12.38 (1H, s, O*H*), 6.39 (1H, br. N*H*), 6.26 (1H, s, ArC*H*), 3.89 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.53 (2H, dt, *J* = 2.5 and 6.5 Hz, NHCH_2CH_2) and 2.91 (2H, t, *J* = 6.5 Hz, NHCH_2CH_2). **^{13}C NMR** (100MHz, $CDCl_3$): δ_C 170.5- (*C*=O), 156.8- (*C*), 155.8- (*C*), 135.1- (*C*), 134.9- (*C*), 105.7- (*C*), 101.7+ (Ar*C*H), 60.65+ (O*C*H_3), 55.9+ (O*C*H_3), 40.2- (NH*C*H_2CH_2) and 28.0- (NHCH_2*C*H_2). Spectroscopic data are consistent with those reported by Judd *et. al.*[93]

Synthesis of 7-hydroxy-8,9-dimethoxy-1,2,3,4-tetrahydrophenanthridin-6(5H)-one 170 and its boron complex 171

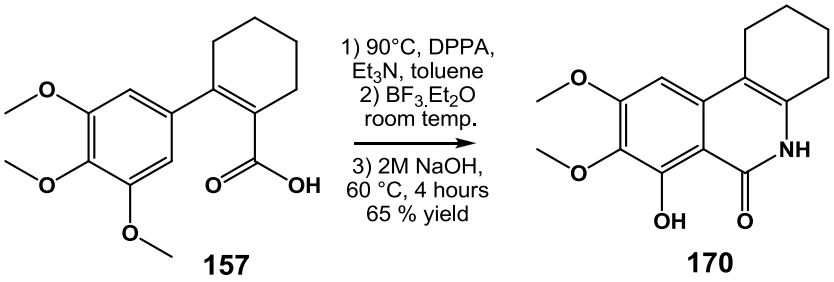


Following a procedure reported by Judd *et al*.,[93] diphenylphosphoryl azide (0.09 mL, 0.40 mmol, 1 eq.) was added dropwise to a stirred solution of **157** (118 mg, 0.40 mmol, 1 eq.) and Et_3N (0.06 mL, 0.40 mmol, 1 eq.), in anydrous toluene (2 mL) at room temperature under N_2. The reaction mixture was then heated with stirring at 90 ^oC for 90 minutes. Most of the solvent was removed under reduced pressure to afford a mobile oil and the flask was cooled to 0 ^oC under N_2. $BF_3.Et_2O$ (1 mL) was added dropwise to the rapidly stirred reaction mixture. The reaction mixture was warmed to room temperature and left to stir for 16 hours. The reaction mixture was quenched with aq. 1M NaOH (to pH 10) and EtOAc (10 mL) was added. The rapidly stirred mixture was heated to 50 ^oC for 1 hour, dissolving all the crude material. The mixture was cooled to room temperature, the layers were separated and the aqueous fraction was further extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried on $NaSO_4$ and filtered and the solvent was removed to give 248 mg of the crude product. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) EtOAc gradient column], **170** (23 mg, 21 % yield) and **171** (50 mg, 39 % yield) were isolated as white solids.

Lactam Product 170 m.p. >220 ^oC. **^{1}H NMR** (400 MHz, $CDCl_3$): δ_H 12.96 (1H, br.s. N*H*), 10.63 (1H, br.s. O*H*), 6.43 (1H, s, ArC*H*), 3.96 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 2.64-2.57 (4H, m, allylic-CH_2) and 1.87-1.85 (4H, m, $(CH_2)_2$). **^{13}C NMR** (100MHz, $CDCl_3$): δ_C 165.6- (*C*=O), 158.2- (*C*), 154.5- (*C*), 135.9- (*C*), 134.4- (*C*), 133.8- (*C*), 111.2- (*C*), 106.0- (*C*), 93.8+.(Ar*C*H), 60.6+ (O*C*H_3), 55.8+ (O*C*H_3), 27.0- (*C*H_2), 23.3- (*C*H_2), 22.4- (*C*H_2) and 21.8- (*C*H_2). **MS** *m/z* (+ESI) 276 (100 %, MH^+) and 298 (38 %, MNa^+). **HRMS** (+ESI) Found MNa^+ 298.1051, $C_{15}H_{17}NNaO_4$ requires *MNa* 298.1055. **IR** ν_{max}(liquid film): 3400 (OH, NH), 2990 (CH) and 1649 (C=O). **Rf** (100 % EtOAc) 0.5.

Boron complex[93] **171** ^{1}**H NMR** (400 MHz, $CDCl_3$): δ_H 13.47 (1H, br.s. N*H*), 6.65 (1H, s, ArC*H*), 3.89 (3H, s, OCH_3), 3.67 (3H, s, OCH_3), 2.63 (2H, br.s. allylic-CH_2), 2.62 (2H, br.s. allylic-CH_2) and 1.72 (4H, br.s. (CH_2)$_2$).

Synthesis of 7-hydroxy-8,9-dimethoxy-1,2,3,4-tetrahydrophenanthridin-6(*5H*)-one 170



157 **170**

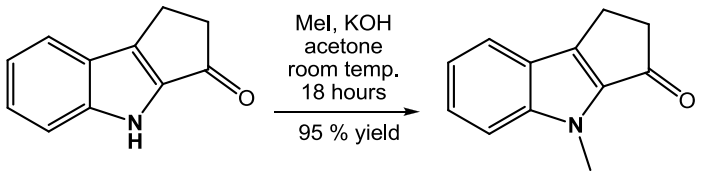
Following a procedure reported by Judd *et al*.,[93] diphenylphosphoryl azide (0.17 mL, 0.81 mmol, 1 eq.) was added dropwise to a stirred solution of **157** (238 mg, 0.81 mmol, 1 eq.) and Et_3N (0.11 mL, 0.81 mmol, 1 eqin anhydrous toluene (4 mL) at room temperature under N_2. The reaction mixture was then heated with stirring at 90 ^oC for 90 minutes. Most of the solvent was removed under reduced pressure to afford a mobile oil and the flask was cooled to 0 ^oC under N_2. $BF_3.Et_2O$ (2 mL) was added dropwise to the rapidly stirred reaction mixture. The reaction mixture was warmed to room temperature and left to stir for 16 hours. The reaction mixture was quenched with aq. 1M NaOH (to pH 10) and EtOAc (10 mL) was added. The rapidly stirred mixture was heated to 60 ^oC for 4 hours, dissolving all the crude material. The mixture was cooled to room temperature, the layers were separated and the aqueous fraction was further extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried on $NaSO_4$ and filtered and the solvent was removed to give 248 mg of the crude product. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], **170** (146 mg, 65 % yield) was isolated as a white solid. Data consistent to that previously reported for lactam **170** (page 211).

7.6. Indanocine chapter

General Procedure 11, Methylation Reaction

Following the procedure reported by Judd *et al.*,[93] MeI (2.4 eq.) was added to a rapidly stirred mixture of KOH (2.9 eq.) and the desired indole (1 eq.) in acetone (10 mL/ 1 mmol). After 18 hours at room temperature, the acetone was removed under reduced pressure. H_2O was added to the residue followed by acidification to pH 1 with 6M $HCl_{(aq.)}$. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were washed with brine, dried on Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired methylated product.

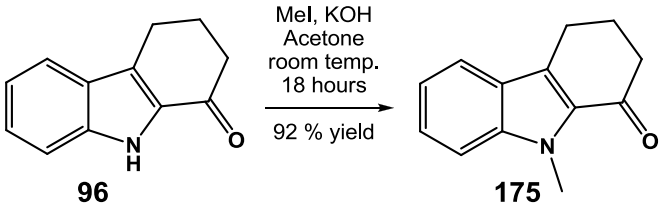
Synthesis of 4-methyl-1,2-dihydrocyclopenta[*b*]indol-3(*4H*)-one 174



98 **174**

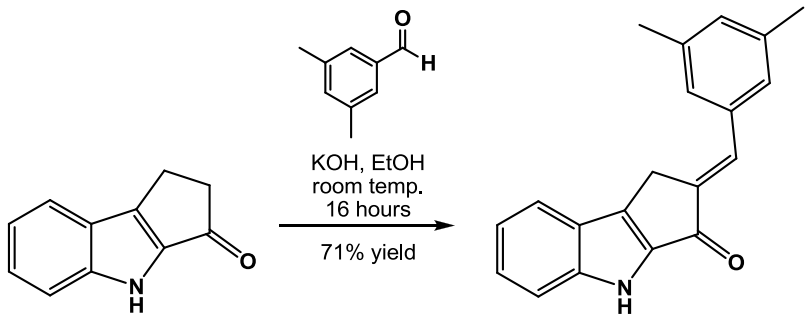
Following **General Procedure 11**,[93] using cyclic ketone **98** on a 6.37 mmol scale, methylated compound **174** (1.12 g, 95 % yield) was isolated as a beige solid, **m.p.** 138-142 ^{o}C [lit.[191] 135.1-136.1 ^{o}C]. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.69 (1H, dt, *J* = 8.0, 1.0 Hz, ArC*H*), 7.41 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.36 (1H, dt, *J* = 8.5, 1.0 Hz, ArC*H*), 7.17 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 3.91 (3H, s, NCH_3), 3.07-3.05 (2H, m, (CO)CH_2CH_2) and 2.99-2.97 (2H, m, (CO)CH_2CH_2). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 194.8- (*C*=O), 145.0- (*C*), 144.8- (*C*), 138.9- (*C*), 126.8+ (Ar*C*H), 123.1- (*C*), 121.7+ (Ar*C*H), 120.2+ (Ar*C*H), 110.9+ (Ar*C*H), 41.5- ((CO)CH_2CH_2), 30.0+ (NCH_3) and 19.6- ((CO)CH_2CH_2). **MS** *m/z* (+ESI) 186 (100 %, MH^+). **HRMS** (+ESI) Found MH^+ 186.0914, $C_{12}H_{12}NO$ requires *MH* 186.0919 and found MNa^+ 208.0736, $C_{12}H_{11}NNaO$ requires *MNa* 208.0738. **IR** ν_{max}(liquid film): 3033 (CH), 1681 (C=O). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C) 0.4. Spectroscopic data are consistent with those reported by Maertens *et al.*[92]

Synthesis of 9-methyl-2,3,4,9-tetrahydro-*1H*-carbazol-1-one 175



Following **General Procedure 11**,[93] using cyclic ketone **96** on a 1.95 mmol scale, methylated compound **175** (358 mg, 92 % yield) was isolated as a beige solid, **m.p.** 96-99 ^{o}C [lit.[192] 100-101 ^{o}C]. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.69 (1H, dt, *J* = 8.0, 1.0 Hz, ArC*H*), 7.44 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.38 (1H, dt, *J* = 8.5, 1.0 Hz, ArC*H*), 7.18 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 4.11 (3H, s, NCH_3), 3.06 (2H, t, *J* = 6.0 Hz, (CO)$CH_2CH_2CH_2$), 2.69 (2H, t, *J* = 6.0 Hz, (CO)$CH_2CH_2CH_2$) and 2.26 (2H, quint, *J* = 6.0 Hz, (CO)$CH_2CH_2CH_2$). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 192.3- (*C*=O), 139.7- (*C*), 130.5- (*C*), 129.2- (*C*), 126.7+ (Ar*C*H), 124.8- (*C*), 121.3+ (Ar*C*H), 120.0+ (Ar*C*H), 110.3+ (Ar*C*H), 40.0- ((CO)$CH_2CH_2CH_2$), 31.5+ (NCH_3), 24.8- ((CO)$CH_2CH_2CH_2$) and 21.9- ((CO)$CH_2CH_2CH_2$). **MS** *m/z* (+ESI) 200 (100 %, MH^+) and 222 (29 %, MNa^+). **HRMS** (+ESI) Found MH^+ 200.1068, $C_{13}H_{14}NO$ requires *MH* 200.1075 and found MNa^+ 222.0886, $C_{13}H_{13}NNaO$ requires *MNa* 222.0894. **IR** ν_{max}(liquid film): 3031 (CH) and 1662 (C=O). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C) 0.8. Spectroscopic data are consistent with those reported by Maertens *et al.*[92]

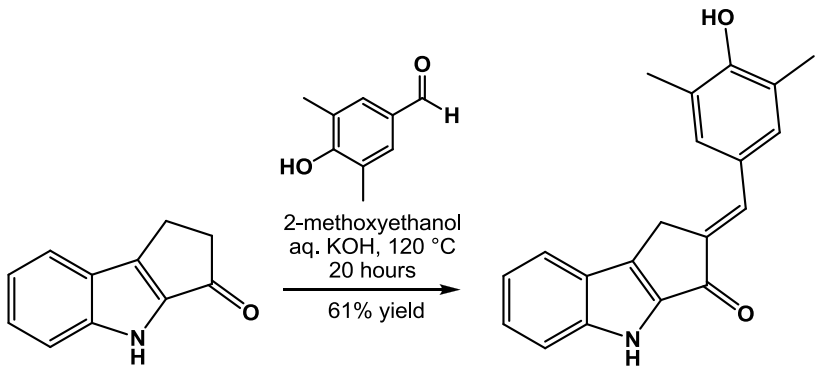
Synthesis of *(E)*-2-(3,5-dimethylbenzylidene)-1,2-dihydrocyclopenta[b]indol-3(4H)-one 176



98 **176**

A mixture of ketone **98** (171 mg, 1 mmol, 1 eq.) and 3,5-dimethylbenzaldehyde (0.15 mL, 1.1 mmol, 1.1 eq.) were treated with 4 % (w/v) ethanolic KOH (10 mL) and stirred at room temperature for 16 hours.[159] The mixture was cooled in an ice-bath and the solid was filtered and washed with EtOH: H_2O (10 mL: 10 mL). H_2O (20 mL) was added to the filtrate which was then neutralised using AcOH, followed by a second filtration. The solid was again washed with EtOH: H_2O (10 mL: 10 mL) to collect **176** (206 mg, 71 % yield) as a yellow solid, **m.p.** 288-292 ^oC. **^{1}H NMR** (400 MHz; $(CD_3)_2SO$): δ_H 11.90 (1H, br.s, N*H*), 7.80 (1H, d, *J* = 8.0 Hz, ArC*H*), 7.45 (1H, d, *J* = 8.5 Hz, ArC*H*), 7.37-7.33 (3H, m, ArC*H* and Ar'C*H*), 7.29 (1H, br.s, alkene C*H*), 7.14 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.05 (1H, s, Ar'C*H*), 4.04 (2H, s, CH_2) and 2.33 (6H, s, Ar'CH_3). **^{13}C NMR** (100 MHz; $(CD_3)_2SO$): δ_C 181.8- (*C*O), 143.5- (*C*), 140.5- (*C*), 139.9- (*C*), 139.6- (*C*), 138.0- (*C*), 134.9- (*C*), 130.8+ (alkene *C*H), 130.8+ (Ar'*C*H), 128.1+ (Ar*C*H or Ar'*C*H), 126.8+ (Ar'*C*H or Ar*C*H), 122.7- (*C*), 121.7+ (Ar*C*H), 120.3+ (Ar*C*H), 113.6+ (Ar*C*H), 26.3- (*C*H_2) and 20.9+ (Ar'*C*H_3). **MS** *m/z* (+ESI) 288 (100 %, MH^+) and 310 (14 %, MNa^+). **HRMS** (+ESI) Found MH^+ 288.1369, $C_{20}H_{18}NO$ requires *MH* 288.1388 and found MNa^+ 310.1190, $C_{20}H_{17}NNaO$ requires *MNa* 310.1208. **IR** ν_{max}(liquid film): 3464 (NH), 3028 (CH), 1678 (C=O) and 1627 (C=C). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^oC) 0.5. **Analysis** (Found: H, 5.92; N, 4.86. $C_{20}H_{17}NO$ requires H, 5.96; N, 4.87 %).

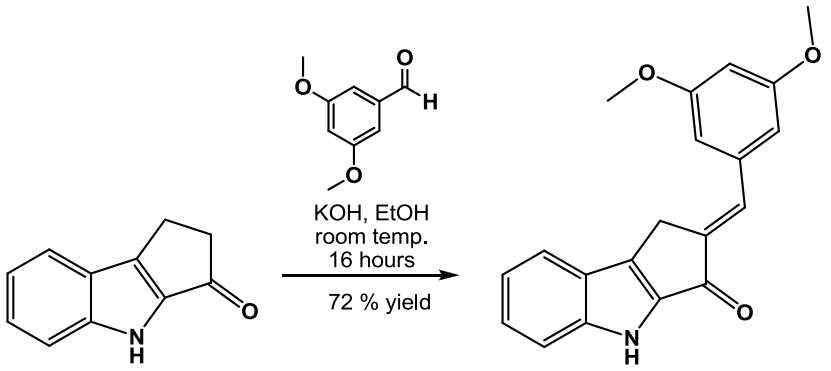
Synthesis of *(E)*-2-(4-hydroxy-3,5-dimethylbenzylidene)-1,2-dihydrocyclopenta[*b*] indol-3(*4H*)-one 177



98 **177**

Ketone **98** (128 mg, 0.75 mmol, 1 eq.) and 3,5-dimethyl-4-hydroxybenzaldehyde (123 mg, 0.82 mmol, 1.1 eq.) were dissolved in 2-methoxyethanol (1 mL), followed by the addition of >99 % aqueous KOH solution (0.5 mL). The reaction mixture was heated at 120 ^{o}C for 20 hours. The mixture was cooled in an ice bath and the solid was filtered and washed with EtOH: H_2O (10 mL: 10 mL). H_2O (15 mL) was added to the filtrate which was then neutralised using AcOH, followed by a second filtration. The solid was again washed with EtOH: H_2O (10mL: 10mL) to collect **177** (139 mg, 61 % yield) as a yellow solid, **m.p.** > 330 ^{o}C. **^{1}H NMR** (400 MHz; $(CD_3)_2SO$): δ_H 11.80 (1H, br.s, N*H*), 8.78 (1H, br.s, O*H*), 7.79 (1H, d, *J* = 8.0 Hz, ArC*H*), 7.44 (1H, d, *J* = 8.5 Hz, ArC*H*), 7.35-7.32 (3H, m, Ar'C*H* and ArC*H*), 7.22 (1H, br.s, alkene C*H*), 7.13 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 3.97 (2H, s, CH_2) and 2.22 (6H, s, Ar'CH_3). **^{13}C NMR** (100 MHz; $(CD_3)_2SO$): δ_C 182.1- (*C*O), 155.0- (*C*), 143.3- (*C*), 140.8- (*C*), 138.8- (*C*), 136.7- (*C*), 131.2+ (alkene *C*H), 131.0+ (Ar'*C*H), 126.5+ (Ar*C*H), 126.1- (*C*), 124.7- (*C*), 121.5+ (Ar*C*H), 120.2+ (Ar*C*H), 113.6+ (Ar*C*H), 26.3- (*C*H_2) and 16.6+ (Ar'*C*H_3). **MS** *m/z* (+ESI) 304 (100 %, MH^+) and 326 (11 %, MNa^+). **HRMS** (+ESI) Found MH^+ 304.1334, $C_{20}H_{18}NO_2$ requires *MH* 304.1338 and found MNa^+ 326.1170, $C_{20}H_{17}NNaO_2$ requires *MNa* 326.1157. **IR** ν_{max}(liquid film): 3605 (OH), 3463 (NH), 3036 (CH), 1677 (C=O) and 1601 (C=C). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C) 0.2.

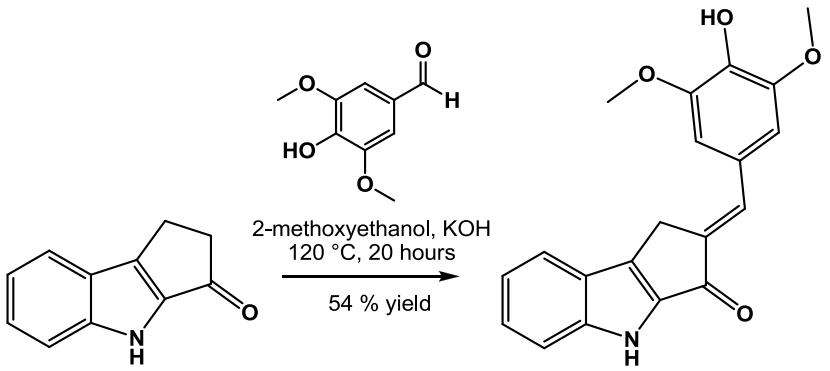
Synthesis of *(E)*-2-(3,5-dimethoxybenzylidene)-1,2-dihydrocyclopenta[*b*]indol-3(*4H*)-one 178



98 **178**

A mixture of ketone **98** (171 mg, 1 mmol, 1eq.) and 3,5-dimethoxybenzaldehyde (183 mg, 1.1 mmol, 1.1 eq.) were treated with 4 % (w/v) ethanolic KOH (10 mL) and stirred at room temperature for 16 hours.[159] The mixture was cooled in an ice-bath and the solid was filtered and washed with EtOH: H_2O (10 mL: 10 mL). H_2O (15 mL) was added to the filtrate which was then neutralised using AcOH, followed by a second filtration. The solid was again washed with EtOH: H_2O (10 mL: 10 mL) to collect **178** (232 mg, 72 % yield) as a yellow solid, **m.p.** >220 ^oC. **^{1}H NMR** (400 MHz; $(CD_3)_2SO$): δ_H 11.86 (1H, br.s, N*H*), 7.80 (1H, d, *J* = 8.0 Hz, ArC*H*), 7.45 (1H, d, *J* = 8.5 Hz, ArC*H*), 7.36 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.30 (1H, m, alkene C*H*), 7.14 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 6.90 (2H, d, *J* = 2.0 Hz, Ar'C*H*), 6.57 (1H, t, *J* = 2.0 Hz, Ar'C*H*), 4.03 (2H, d, *J* = 1.5 Hz, CH_2) and 3.81 (6H, s, Ar'OCH_3). **^{13}C NMR** (100 MHz; $(CD_3)_2SO$): δ_C 181.7- (*C*O), 160.7- (*C*), 143.5- (*C*), 140.7- (*C*), 140.4- (*C*), 139.8- (*C*), 136.8- (*C*), 130.6+ (alkene *C*H), 126.9+ (Ar*C*H), 122.6- (*C*), 121.8+ (Ar*C*H), 120.3+ (Ar*C*H), 113.6+ (Ar*C*H), 108.3+ (Ar'*C*H), 101.3+ (Ar'*C*H), 55.4+ (Ar'O*C*H_3) and 26.2- (CH_2). **MS** *m/z* (+ESI) 320 (100 %, MH^+) and 342 (9 %, MNa^+). **HRMS** (+ESI) Found MH^+ 320.1281, $C_{20}H_{18}NO_3$ requires *MH* 320.1287. **IR** ν_{max}(liquid film): 3464 (NH), 3026 (CH), 1674 (C=O) and 1624 (C=C). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^oC) 0.3.

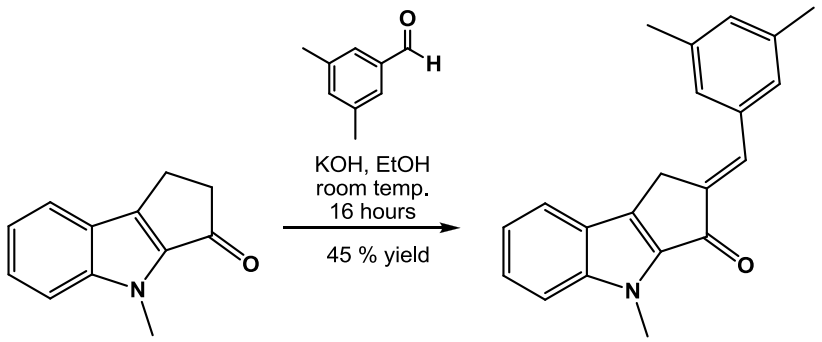
Synthesis of *(E)*-2-(3,5-dimethoxybenzylidene)-1,2-dihydrocyclopenta[b]indol-3(4H)-one 179



98 **179**

Ketone **98** (171 mg, 1 mmol, 1 eq.) and 3,5-dimethoxy-4-hydroxybenzaldehyde (200 mg, 1.1 mmol, 1.1 eq.) were dissolved in 2-methoxyethanol (1 mL), followed by the addition of >99 % aqueous KOH solution (0.5 mL). The reaction mixture was heated at 120 ^{o}C for 20 hours. The mixture was cooled in an ice-bath and the solid was filtered and washed with EtOH: H_2O (10 mL: 10 mL). H_2O (15 mL) was added to the filtrate which was then neutralised using AcOH, followed by a second filtration. The solid was again washed with EtOH: H_2O (10 mL: 10 mL) to collect **179** (182 mg, 54 % yield) as a yellow solid, **m.p.** 237-239 ^{o}C. **^{1}H NMR** (400 MHz; $(CD_3)_2SO$): δ_H 11.86 (1H, br.s. N*H*), 8.94 (1H, s, O*H*), 7.80 (1H, d, *J* = 8.0 Hz, ArC*H*), 7.55 (1H, d, *J* = 8.5 Hz, ArC*H*), 7.40 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.29 (1H, br.s, alkene C*H*), 7.17 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.02 (2H, s, Ar'C*H*), 4.03 (2H, s, CH_2) and 3.85 (6H, s, Ar'OCH_3). **^{13}C NMR** (100 MHz; $(CD_3)_2SO$): δ_C 182.0- (*C*=O), 148.1- (*C*), 143.3- (*C*), 140.7- (*C*), 139.0- (*C*), 137.7- (*C*), 137.3- (C), 131.6+ (alkene *C*H), 126.6+ (Ar*C*H), 125.4- (*C*), 122.7- (*C*), 121.6+ (Ar*C*H), 120.2+ (Ar*C*H), 113.6+ (Ar*C*H), 108.5+ (Ar'*C*H), 56.2+ (Ar'O*C*H_3) and 26.1- (CH_2). **MS** *m/z* (+ESI) 350 (100 %, MH^+) and 372 (12 %, MNa^+). **HRMS** (+ESI) Found MH^+ 350.1376, $C_{21}H_{20}NO_4$ requires *MH* 350.1392 and found MNa^+ 372.1189, $C_{21}H_{19}NNaO_4$ requires *MNa* 372.1212. **IR** ν_{max}(liquid film): 3529 (OH), 3011 (CH), 1673 (C=O) and 1624 (C=C). **Rf** (70 % EtOAc in light petroleum (b.p. 40-60 ^{o}C) 0.6.

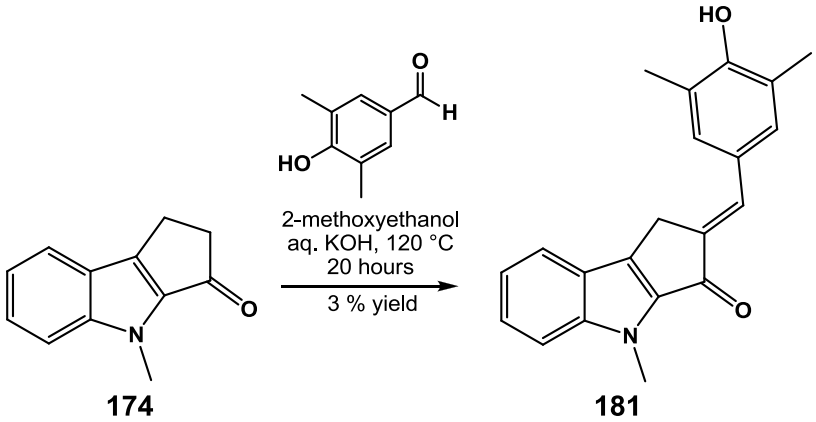
Synthesis of *(E)*-2-(3,5-dimethylbenzylidene)-4-methyl-1,2-dihydrocyclopenta[*b*] indol-3(*4H*)-one 180



174 **180**

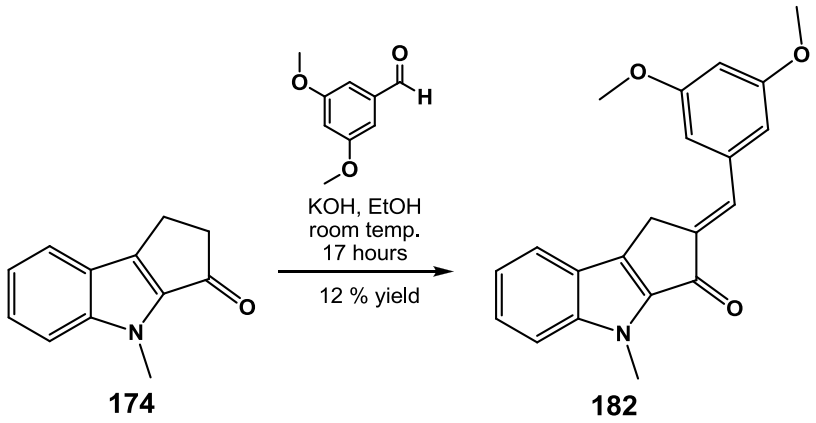
A mixture of ketone **174** (185 mg, 1 mmol, 1 eq.) and 3,5-dimethylbenzaldehyde (0.15 mL, 1.1 mmol, 1.1 eq.) were treated with 4 % (w/v) ethanolic KOH (10 mL) and stirred at room temperature for 16 hours.[159] The mixture was cooled in an ice-bath and the solid was filtered and washed with EtOH: H_2O (10 mL: 10 mL). H_2O (20 mL) was added to the filtrate which was then neutralised using AcOH, followed by a second filtration. The solid was again washed with EtOH: H_2O (10 mL: 10 mL) to collect **180** (135 mg, 45 % yield) as a yellow solid, **m.p.** 174-177 ^oC. **^{1}H NMR** (400 MHz; $(CD_3)_2SO$): δ_H 7.76 (1H, dt, *J* = 8.0, 1.0 Hz, ArC*H*), 7.47 (1H, t, *J* = 1.5 Hz, alkene C*H*), 7.42 (1H, ddd, *J* = 8.5, 6.5, 1.0 Hz, ArC*H*), 7.38 (1H, dt, *J* = 8.5, 1.0 Hz, ArC*H*), 7.28 (2H, s, Ar'C*H*), 7.20 (1H, ddd, *J* = 8.0, 6.5, 1.0 Hz, ArC*H*), 7.02 (1H, s, Ar'C*H*), 4.00 (3H, s, NCH_3), 3.95 (2H, d, *J* = 1.5 Hz, CH_2) and 2.39 (6H, s, Ar'CH_3). **^{13}C NMR** (100 MHz; $(CD_3)_2SO$): δ_C 183.4- (*C*=O), 144.6- (*C*), 140.9- (*C*), 139.8- (*C*), 138.6- (*C*), 138.3- (*C*), 135.5- (*C*), 132.0+ (alkene *C*H), 131.0+ (Ar'*C*H), 128.3+ (Ar'*C*H), 126.8+ (Ar*C*H), 123.0- (*C*), 121.9+ (Ar*C*H), 120.5+ (Ar*C*H), 111.0+ (Ar*C*H), 30.3+ (N*C*H_3), 26.5- (*C*H_2) and 21.4+ (Ar'*C*H_3). **MS** *m/z* (+ESI) 302 (100 %, MH^+). **HRMS** (+ESI) Found MH^+ 302.1535, $C_{21}H_{20}NO$ requires *MH* 302.1545 and found MNa^+ 324.1353, $C_{21}H_{19}NNaO$ requires *MNa* 324.1364. **IR** ν_{max}(liquid film): 3029 (CH), 1678 (C=O) and 1628 (C=C). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^oC) 0.8. **Analysis** (Found: H, 6.47; N, 4.29. $C_{21}H_{19}NO$ requires H, 6.35; N, 4.65 %).

Synthesis of *(E)*-2-(4-hydroxy-3,5-dimethylbenzylidene)-4-methyl-1,2-dihydrocyclopenta[b]indol-3(4H)-one 181



Ketone **174** (226 mg, 1.22 mmol, 1 eq.) and 3,5-dimethyl-4-hydroxybenzaldehyde (201 mg, 1.34 mmol, 1.1 eq.) were dissolved in 2-methoxyethanol (1.2 mL), followed by the addition of >99 % aqueous KOH solution (0.6 mL). The reaction mixture was heated at 120 ^{o}C for 20 hours. The mixture was cooled, H_2O (10 mL) was added and the mixture was neutralised using AcOH. The product was extracted using $CHCl_3$ (3 x 20 mL), the combined organic layers were washed with brine (20 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], product **181** (13 mg, 3 % yield) was isolated as yellow crystals, **m.p.** 221-224 ^{o}C. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.77 (1H, dt, *J* = 8.0, 1.0 Hz, ArC*H*), 7.42-7.40 (3H, m, alkene C*H*, ArC*H*, ArC*H*), 7.34 (2H, s, Ar'C*H*), 7.20 (1H, ddd, *J* = 8.0, 6.5, 1.0 Hz, ArC*H*), 4.86 (1H, s, O*H*), 4.02 (3H, s, NCH_3), 3.95 (2H, d, *J* = 1.5 Hz, CH_2) and 2.32 (6H, s, Ar'C*H*$_3$). **MS** *m/z* (+ESI) 318 (100 %, MH^+) and 340 (34 %. MNa^+). **HRMS** Found MH^+ 318.1499, $C_{21}H_{20}NO_2$ requires *MH* 318.1494. **IR** ν_{max}(liquid film): 3387 (OH), 3022 (CH), 1671 (C=O) and 1600 (C=C). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C) 0.5.

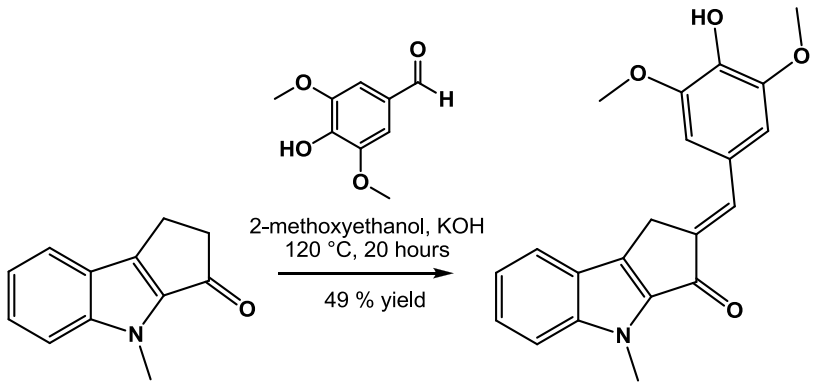
Synthesis of *(E)*-2-(3,5-dimethoxybenzylidene)-4-methyl-1,2-dihydrocyclopenta[b] indol-3(4H)-one 182



174 182

A mixture of ketone **174** (185 mg, 1 mmol, 1 eq.) and 3,5-dimethoxybenzaldehyde (183 mg, 1.1 mmol, 1.1 eq.) were treated with 4 % (w/v) ethanolic KOH (10 mL) and stirred at room temperature for 17 hours.[159] The mixture was cooled in an ice-bath and the solid was filtered and washed with EtOH: H_2O (10 mL: 10 mL). H_2O (15 mL) was added to the filtrate which was then neutralised using AcOH, followed by a second filtration. The solid was again washed with EtOH: H_2O (10 mL: 10 mL) to collect 301 mg of crude product. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], product **182** (40 mg, 12 % yield) was isolated as a yellow solid, **m.p.** 178-180 ^oC. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.73 (1H, dt, *J* = 8.0, 1.0 Hz, ArC*H*), 7.44-7.37 (3H, m, ArC*H* and alkene C*H*), 7.20 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 6.81 (2H, d, *J* = 2.0 Hz, Ar'C*H*), 6.51 (1H, t, *J* = 2.0 Hz, Ar'C*H*), 4.01 (3H, s, NCH_3), 3.97 (2H, d, *J* = 1.5 Hz, CH_2) and 3.86 (6H, s, Ar'OCH_3). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 183.1- (*C*=O), 161.0- (*C*), 144.7- (*C*), 140.8- (*C*), 140.7- (*C*), 138.7- (*C*), 137.3- (*C*), 131.6+ (alkene *C*H), 127.0+ (Ar*C*H), 122.9- (*C*), 121.9+ (Ar*C*H), 120.6+ (Ar*C*H), 111.0+ (Ar*C*H), 108.5+ (Ar'*C*H), 101.2+ (Ar'*C*H), 55.5+ (Ar'O*C*H_3), 30.3 (N*C*H_3) and 26.4- (*C*H_2). **MS** *m/z* (+ESI) 334 (100 %, MH^+) and 356 (8 %, MNa^+). **HRMS** (+ESI) Found MH^+ 334.1445, $C_{21}H_{20}NO_3$ requires *MH* 334.1443 and found MNa^+ 356.1270, $C_{21}H_{19}NNaO_3$ requires *MNa* 356.1263. **IR** ν_{max}(liquid film): 3014 (CH), 1677 (C=O) and 1592 (C=C). **Rf** (40 % EtOAc in light petroleum (b.p. 40-60 ^oC) 0.4

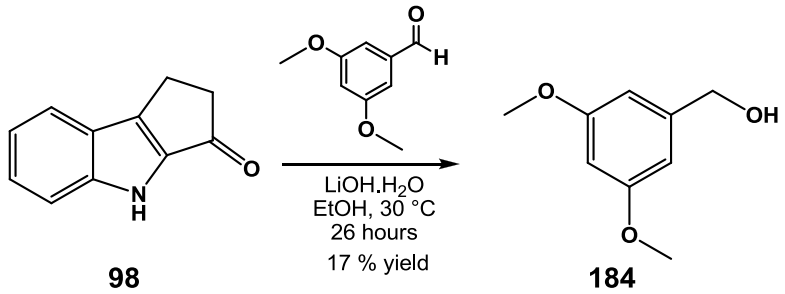
Synthesis of *(E)*-2-(4-hydroxy-3,5-dimethoxybenzylidene)-4-methyl-1,2-dihydro-cyclopenta[b] indol-3(4H)-one 183



174 183

Ketone **174** (185 mg, 1 mmol, 1 eq.) and 3,5-dimethoxy-4-hydroxybenzaldehyde (200 mg, 1.1 mmol, 1.1 eq.) were dissolved in 2-methoxyethanol (1 mL), followed by the addition of >99 % aqueous KOH solution (0.5 mL). The reaction mixture was heated at 120 ^{o}C for 20 hours. The mixture was cooled in an ice-bath and the solid filtered and washed with EtOH: H_2O (10 mL: 10 mL). H_2O (15 mL) was added to the filtrate which was then neutralised using AcOH, followed by a second filtration. The solid was again washed with EtOH: H_2O (10 mL: 10 mL) to collect **183** (171 mg, 49 % yield) as a yellow solid, **m.p.** 191-194 ^{o}C. **^{1}H NMR** (400 MHz; $(CD_3)_2SO$): δ_H 8.97 (1H, br.s. O*H*), 7.80 (1H, d, *J* = 8.0 Hz, ArC*H*), 7.55 (1H, d, *J* = 8.0 Hz, ArC*H*), 7.40 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.29 (1H, br.s, alkene C*H*), 7.17 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.02 (2H, s, Ar'C*H*), 4.00 (2H, s, CH_2), 3.91 (3H, s, NCH_3) and 3.85 (6H, s, Ar'OCH_3). **^{13}C NMR** (100 MHz; $(CD_3)_2SO$): δ_C 182.3- (*C*=O), 148.1- (*C*), 144.1- (*C*), 140.2- (*C*), 137.8- (*C*), 137.8- (*C*), 137.3- (C), 131.7+ (alkene *C*H), 126.6+ (Ar*C*H), 125.2- (*C*), 122.3- (*C*), 121.8+ (Ar*C*H), 120.3+ (Ar*C*H), 111.5+ (Ar*C*H), 108.5+ (Ar'*C*H), 56.2+ (Ar'O*C*H_3), 30.0 (NCH_3) and 25.8- (CH_2). **MS** *m/z* (+ESI) 336 (100 %, MH^+) and 358 (12 %, MNa^+). **HRMS** (+ESI) Found MH^+ 336.1228, $C_{20}H_{18}NO_4$ requires *MH* 336.1236 and found MNa^+ 358.1050, $C_{20}H_{17}NNaO_4$ requires *MNa* 358.1055. **IR** ν_{max}(liquid film): 3690 (OH), 3464 (NH), 1677 (C=O) and 1627 (C=C).

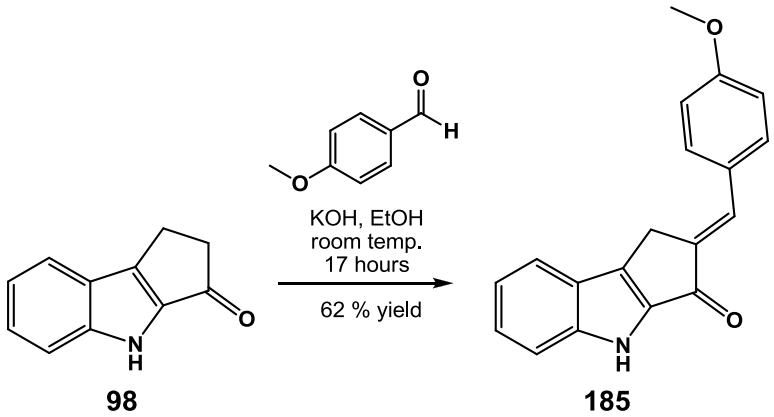
Synthesis of (3,5-dimethoxyphenyl)methanol 184



98 **184**

Ketone **98** (255 mg, 1.49 mmol, 1 eq.) and $LiOH.H_2O$ (188 mg, 4.48 mmol, 3 eq.) in EtOH (2 mL) were stirred for 10 minutes, followed by the addition of 3,5-dimethoxybenzaldehyde (494 mg, 2.97 mmol, 2 eq.). The reaction mixture was stirred at 30 ^oC for 26 hours and the solvent was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], product **184** (84 mg, 17 % yield) was isolated as a pale yellow solid, **m.p.** 45-48 ^oC [lit.[193] 46-48 ^oC]. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ_H 6.56 (2H, d, *J* = 2.5 Hz, ArC*H*), 6.42 (1H, t, *J* = 2.5 Hz, ArC*H*), 4.67 (2H, s, CH_2), 3.83 (6H, s, OCH_3) and 1.80 (1H, m, O*H*). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 161.1- (*C*), 143.4- (*C*), 104.6+ (Ar*C*H), 99.7+ (Ar*C*H), 65.4- (CH_2) and 55.4+ (O*C*H_3). Spectroscopic data are consistent with those reported by Lesch *et al.*[194]

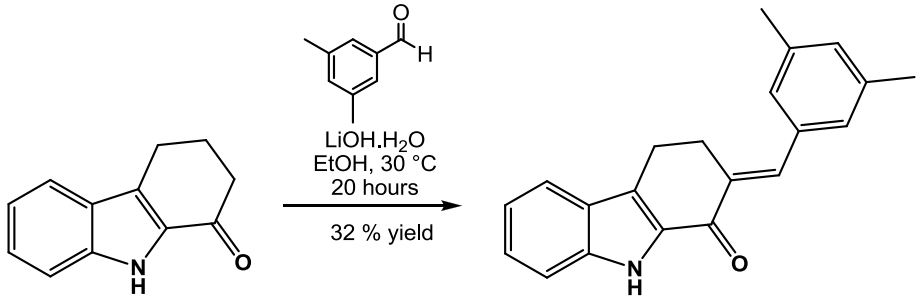
Synthesis of *(E)*-2-(4-methoxybenzylidene)-1,2-dihydrocyclopenta[b]indol-3(4h)-one 185



98 **185**

A mixture of ketone **98** (171 mg, 1 mmol, 1 eq.) and 4-methoxybenzaldehyde (0.12 mL, 1 mmol, 1 eq.) were treated with 4 % (w/v) ethanolic KOH (10 mL) and stirred at room temperature for 17 hours.[159] The mixture was cooled in an ice-bath and the solid was filtered and washed with EtOH: H_2O (10 mL: 10 mL). H_2O (20 mL) was added to the filtrate which was then neutralised using AcOH, followed by a second filtration. The solid was again washed with EtOH: H_2O (10 mL: 10 mL) to collect **185** (181 mg, 62 % yield) as a yellow solid, **m.p.** 278-281 ^oC [lit.[195] 262 ^oC]. **^{1}H NMR** (400 MHz; $(CD_3)_2SO$): δ_H 11.84 (1H, br.s, N*H*), 7.76 (1H, d, *J* = 8.0 Hz, ArC*H*), 7.70 (2H, d, *J* = 8.5 Hz, Ar'C*H*), 7.45 (1H, d, *J* = 8.5 Hz, ArC*H*), 7.34 (1H, t, *J* = 7.0 Hz, ArC*H*), 7.33 (1H, br.s, alkene C*H*), 7.14 (1H, t, *J* = 7.5 Hz, ArC*H*), 7.03 (2H, d, *J* = 8.5 Hz, Ar'C*H*), 3.99 (2H, s, CH_2) and 3.81 (3H, s, Ar'OCH_3). **^{13}C NMR** (100 MHz; $(CD_3)_2SO$): δ_C 182.0- (*C*=O), 160.2- (*C*), 143.4- (*C*), 140.7- (C), 139.1- (C), 137.8- (C), 132.1+ (Ar'*C*H), 130.4+ (alkene *C*H), 127.6- (*C*), 126.6+ (Ar*C*H), 122.7- (*C*), 121.5+ (Ar*C*H), 120.2+ (Ar*C*H), 114.5+ (Ar'*C*H), 113.6+ (Ar*C*H), 55.3+ (Ar'O*C*H_3) and 26.3- (CH_2). **MS** *m/z* (+ESI) 290 (100 %, MH^+) and 312 (6 %, MNa^+). **HRMS** (+ESI) Found MH^+ 290.1184, $C_{19}H_{16}NO_2$ requires *MH* 290.1181 and found MNa^+ 312.1000, $C_{19}H_{15}NNaO_2$ requires *MNa* 312.1000. **IR** ν_{max}(liquid film): 3464 (NH), 3024 (CH), 1673 (C=O) and 1602 (C=C). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^oC) 0.3. **Analysis** (Found: H, 4.90; N, 4.60. $C_{19}H_{15}NO_2$ requires H, 5.23; N, 4.84 %).

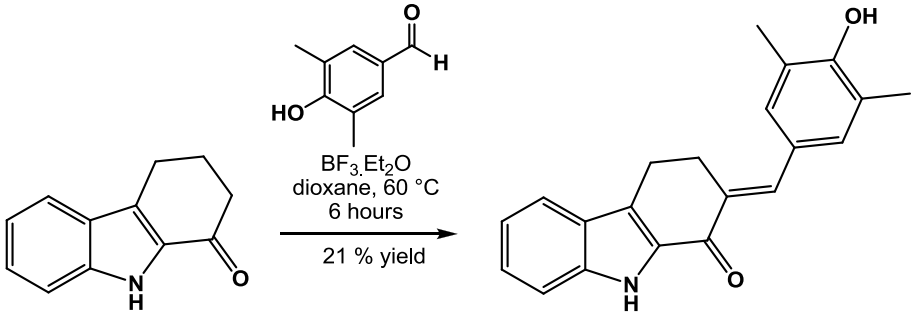
Synthesis of *(E)*-2-(3,5-dimethyl benzylidene)-2,3,4,9-tetrahydro-1H-carbazol-1-one 186



96 **186**

Following a procedure reported by Chakraborti *et al.*[156] ketone **96** (203 mg, 1.1 mmol, 1 eq.) and LiOH.H_2O (51 mg, 1.21 mmol, 1.1 eq.) in EtOH (3 mL) were stirred for 10 minutes, followed by the addition of 3,5-dimethylbenzaldehyde (0.15 mL, 1.1 mmol, 1.1 eq.). The reaction mixture was stirred at 30 ^{o}C for 21 hours. The solvent was removed under reduced pressure and the yellow residue was diluted with H_2O (10 mL) and EtOAc (10 mL). The mixture was neutralised with 6M $HCl_{(aq.)}$ followed by extraction with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (10 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], product **186** (95 mg, 32 % yield) was isolated as a pale yellow solid, **m.p.** 209-211 ^{o}C (recrystallised from EtOAc). **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 9.21 (1H, br.s, N*H*), 7.80 (1H, br.s, alkene C*H*), 7.70 (1H, dd, *J* = 8.0, 1.0 Hz, ArC*H*), 7.48 (1H, dt, *J* = 8.5, 1.0 Hz, ArC*H*), 7.41 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.20 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.11 (2H, s, Ar'C*H*), 7.04 (1H, s, Ar'C*H*), 3.30 (2H, td, *J* = 6.5, 1.5 Hz, CH_2CH_2C=CH), 3.11 (2H, t, *J* = 6.5 Hz, CH_2CH_2C=CH), 2.41 (6H, s, Ar'CH_3). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 181.1- (*C*=O), 138.6- (*C*), 138.0- (*C*), 136.1- (*C*), 136.0- (*C*), 135.7+ (alkene *C*H), 132.4- (*C*), 130.1+ (Ar'*C*H), 128.3- (*C*), 127.6+ (Ar'*C*H), 127.2+ (Ar*C*H), 126.0- (*C*), 121.4+ (Ar*C*H), 120.5+ (Ar*C*H), 112.5+ (Ar*C*H), 27.7- (CH_2CH_2C=CH), 21.4+ (Ar'CH_3) 20.9- (CH_2CH_2C=CH). **MS** *m/z* (+ESI) 302 (100 %, MH^+) and 324 (32 %, MNa^+). **HRMS** (+ESI) Found MH^+, 302.1546, $C_{21}H_{20}NO$ requires *MH* 302.1545 and found MNa^+ 324.1363, $C_{21}H_{19}NNaO$ requires *MNa* 324.1364. **IR** ν_{max}(liquid film): 3460 (NH), 3046 (CH), 1651 (C=O) and 1601 (C=C). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^{o}C) 0.8.

Synthesis of *(E)*-2-(4-hydroxy-3,5-dimethylbenzylidene)-2,3,4,9-tetrahydro-1H-carbazol-1-one 187

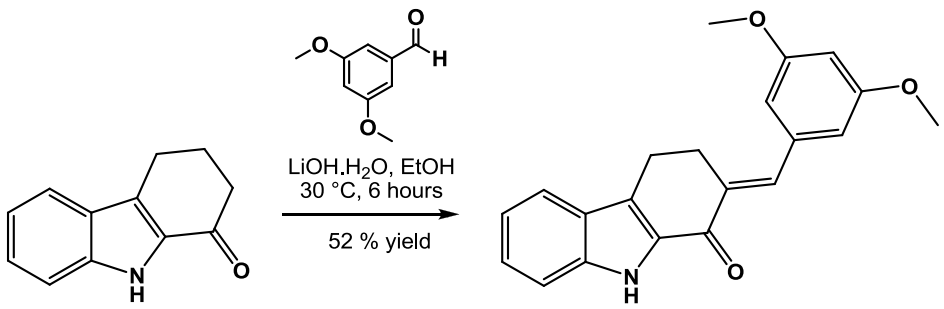


96 **187**

All glassware was dried under N_2 using a heatgun. Ketone **96** (192 mg, 1.04 mmol, 1 eq.) and 3,5-dimethyl-4-hydroxybenzaldehyde (156 mg, 1.04 mmol, 1 eq.) were added to the flask followed by anhydrous dioxane (2 mL). $BF_3{\cdot}Et_2O$ (0.4 mL, 3.12 mmol, 3 eq.) was added slowly and the reaction mixture was heated at 60 ^oC for 6 hours.[160] On cooling, H_2O (60 mL), 2M $NaOH_{(aq.)}$ (20 mL) and EtOAc (60 mL) were added to the reaction mixture. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 60 mL). The combined organic layers were washed with brine (60 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure to collect 343 mg of crude product. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], product **187** (70 mg, 21 % yield) was isolated as a yellow solid, **m.p.** 186-188 ^oC. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 8.80 (1H, br.s, N*H*), 7.73 (1H, br.s, alkene C*H*), 7.70 (1H, dd, *J* = 8.0, 1.0 Hz, ArC*H*), 7.46 (1H, dt, *J* = 8.5, 1.0 Hz, ArC*H*), 7.41 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.20 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.17 (2H, s, Ar'C*H*), 4.84 (1H, br.s, O*H*), 3.32 (2H, td, *J* = 6.5, 1.5 Hz, CH_2CH_2C=CH), 3.11 (2H, t, *J* = 6.5 Hz, CH_2CH_2C=CH) and 2.35 (6H, s, Ar'CH_3). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 181.1- (*C*=O), 152.8- (*C*), 138.4- (*C*), 135.8+ (alkene *C*H), 134.1- (*C*), 132.5- (*C*), 130.7+ (Ar'*C*H), 128.2- (*C*), 127.9- (*C*), 127.0+ (Ar*C*H), 126.0- (*C*), 123.2- (*C*), 121.3+ (Ar*C*H), 120.4+ (Ar*C*H), 112.5+ (Ar*C*H), 27.7- (CH_2CH_2C=CH), 20.8- (CH_2CH_2C=CH) and 16.0+ (Ar'CH_3). **MS** *m/z* (+ESI) 318 (100 %, MH^+) and 340 (19 %, MNa^+). **HRMS** (+ESI) Found MH^+ 318.1486, $C_{21}H_{20}NO_2$ requires *MH* 318.1494 and found MNa^+ 340.1302, $C_{21}H_{19}NNaO_2$ requires *MNa*, 340.1313. **IR** ν_{max}(liquid film): 3605 (OH), 3460 (NH), 3042 (CH), 1648 (C=O) and 1599 (C=C). **Rf** (30 % EtOAc in light petroleum

(b.p. 40-60 ^{o}C) 0.5. **NOESY NMR** Interaction observed between 7.17 (2H, s, Ar'C*H*) and 3.32 (2H, td, *J* = 6.5, 1.5 Hz, CH_2CH_2C=CH) to confirm *E* configuration.

Synthesis of *(E)*-2-(3,5-dimethoxybenzylidene)-2,3,4,9-tetrahydro-1H-carbazol-1-one 188



96 **188**

Method A

A mixture of ketone **96** (260 mg, 1.40 mmol, 1 eq.) and 3,5-dimethoxybenzaldehyde (256 mg, 1.54 mmol, 1.1 eq.) were treated with 4 % (w/v) ethanolic KOH (10 mL) and stirred at room temperature for 16 hours.[159] The mixture was cooled in an ice-bath and the solid was filtered and washed with EtOH: H_2O (10 mL: 10 mL). H_2O (20 mL) was added to the filtrate which was then neutralised using AcOH, followed by a second filtration. The solid was again washed with EtOH: H_2O (10 mL: 10 mL) to collect **188** (158 mg, 34 % yield) as a yellow solid.

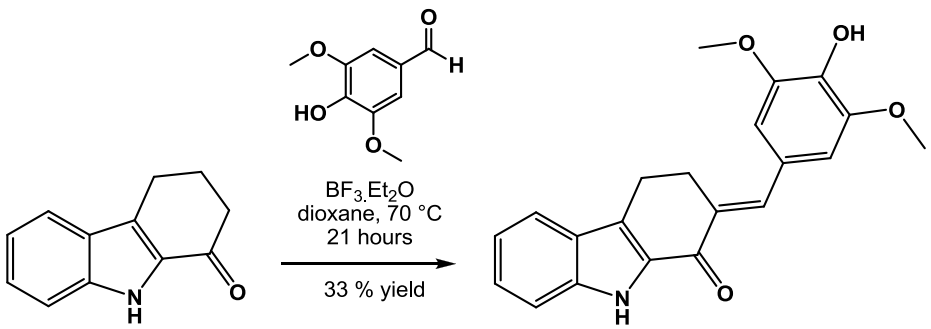
Method B - optimised method

Following a procedure reported by Chakraborti *et al.*[156] ketone **96** (235 mg, 1.27 mmol, 1 eq.) and $LiOH.H_2O$ (133 mg, 3.18 mmol, 2.5 eq.) in EtOH (2 mL) were stirred for 10 minutes, followed by the addition of 3,5-dimethoxybenzaldehyde (318 mg, 1.91 mmol, 1.5 eq.). The reaction mixture was stirred at 30 ^oC for 6 hours. The solvent was removed under reduced pressure and the yellow residue was diluted with water (20 mL) and EtOAc (20 mL). The mixture was neutralised with 6M $HCl_{(aq.)}$ followed by extraction with EtOAc (2 x 10 mL). The combined organic layers were washed with brine (20 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure to collect 456 mg of crude product. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], product **188** (197 mg, **52 % yield**) was isolated as a yellow solid.

m.p. 168-170 ^oC. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 8.96 (1H, br.s, N*H*), 7.76 (1H, br.s, alkene C*H*), 7.70 (1H, dd, *J* = 8.0, 1.0 Hz, ArC*H*), 7.48 (1H, dt, *J* = 8.5, 1.0 Hz, ArC*H*), 7.42 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.20 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 6.63 (2H, dd, *J*

= 2.5, 0.5 Hz, Ar'C*H*), 6.51 (1H, t, *J* = 2.5 Hz, Ar'C*H*), 3.87 (6H, s, Ar'OCH_3), 3.30 (2H, td, *J* = 6.5, 2.0 Hz, CH_2CH_2C=CH) and 3.11 (2H, t, *J* = 6.5 Hz, CH_2CH_2C=CH). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 181.7- (*C*=O), 160.8- (*C*), 140.0- (*C*), 138.1- (*C*), 138.0- (*C*), 135.4- (*C*), 135.3+ (alkene *C*H), 132.3- (*C*), 127.3+ (Ar*C*H), 128.0- (*C*), 127.3+ (Ar*C*H), 121.5+ (Ar*C*H), 120.6+ (Ar*C*H), 112.5+ (Ar*C*H), 107.8+ (Ar'CH), 100.4+ (Ar'CH), 55.4 (OCH_3), 27.7- (CH_2CH_2C=CH) and 20.9- (CH_2CH_2C=CH). **MS** *m/z* (+ESI) 334 (100 %, MH^+) and 356 (9 %, MNa^+). **HRMS** (+ESI) Found MH^+ 334.1433, $C_{21}H_{20}NO_3$ requires *MH* 334.1443 and found MNa^+ 356.1249, $C_{21}H_{19}NNaO_3$ requires *MNa* 356.1263. **IR** ν_{max}(liquid film): 3460 (NH), 3026 (CH), 1652 (C=O) and 1597 (C=C). **Rf** (70 % EtOAc in light petroleum (b.p. 40-60 ^oC) 0.9. **Analysis** (Found: H, 5.74; N, 4.20. $C_{21}H_{19}NO_3$ requires H, 5.74; N, 4.20 %).

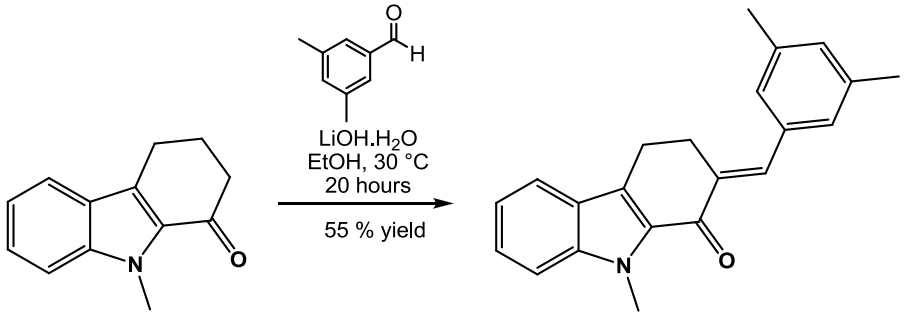
Synthesis of *(E)*-2-(4-hydroxy-3,5-dimethoxybenzylidene)-2,3,4,9-tetrahydro-1H-carbazol-1-one 189



96 **189**

All glassware was dried under N_2 using a heatgun. Ketone **96** (309 mg, 1.67 mmol, 2.5 eq.) and syringaldehyde (122 mg, 0.67 mmol, 1 eq.) were added to the flask followed by anhydrous dioxane (4 mL). $BF_3{\cdot}Et_2O$ (0.25 mL, 2.01 mmol, 3 eq.) was added slowly and the reaction mixture was heated at 70 ^oC for 21 hours.[160] On cooling, H_2O (30 mL), 2M $NaOH_{(aq.)}$ (10 mL) and EtOAc (30 mL) were added to the reaction mixture. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 30 mL). The combined organic layers were washed with brine (30 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure to collect 465 mg of crude product. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) EtOAc gradient column], product **189** (78 mg, 33 % yield) was isolated as a yellow solid, **m.p.** 155-157 ^oC. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 9.09 (1H, br.s, N*H*), 7.78 (1H, br.s, alkene C*H*), 7.70 (1H, dd, *J* = 8.0, 1.0 Hz, ArC*H*), 7.47 (1H, dt, *J* = 8.5, 1.0 Hz, ArC*H*), 7.42 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 7.20 (1H, ddd, *J* = 8.0, 7.0, 1.0 Hz, ArC*H*), 6.76 (2H, s, Ar'C*H*), 5.72 (1H, s, O*H*), 3.97 (6H, s, Ar'OCH_3), 3.35 (2H, td, *J* = 6.5, 1.5 Hz, $CH_2CH_2CH_2C$=CH) and 3.13 (2H, t, *J* = 6.5 Hz, $CH_2CH_2CH_2C$=CH). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 180.7- (*C*O), 147.0- (*C*), 138.5- (*C*), 135.9+ (alkene *C*H), 135.6- (*C*), 134.7- (*C*), 132.4- (*C*), 128.0- (*C*), 127.4- (*C*), 127.2+ (Ar*C*H), 126.0- (*C*), 121.4+ (Ar*C*H), 120.5+ (Ar*C*H), 112.5+ (Ar*C*H), 107.2+ (Ar'*C*H), 56.5+ (Ar'O*C*H_3), 27.7- ($CH_2CH_2CH_2C$=CH) and 20.8- ($CH_2CH_2CH_2C$=CH). **MS** *m/z* (+ESI) 350 (100 %, MH^+). **HRMS** (+ESI) Found MH^+ 350.1394, $C_{21}H_{19}NO_4$ requires *MH*, 350.1400. **IR** ν_{max}(liquid film): 3534 (OH), 3460 (NH), 3033 (CH), 1649 (C=O) and 1612 (C=C). **Rf** (70 % EtOAc in light petroleum (b.p. 40-60 ^oC) 0.7.

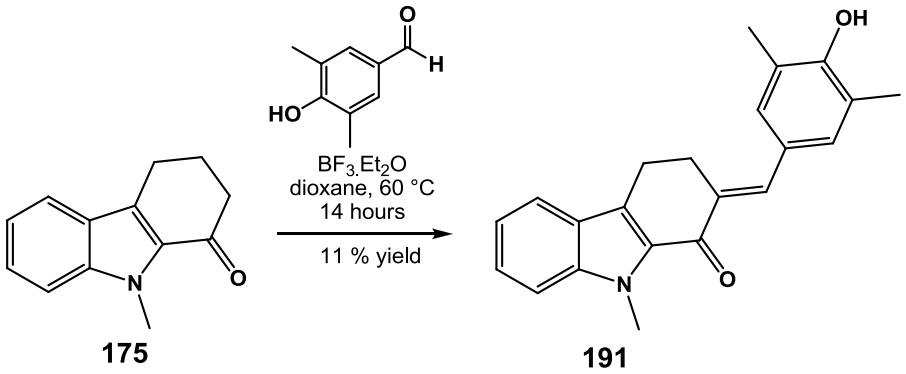
Synthesis of *(E)*-2-(3,5-dimethylbenzylidene)-9-methyl-2,3,4,9-tetrahydro-1H-carbazol-1-one 190



175 **190**

Following a procedure reported by Chakraborti *et al.*[156] ketone **175** (172 mg, 0.86 mmol, 2 eq.) and $LiOH.H_2O$ (54 mg, 1.29 mmol, 3 eq.) in EtOH (2 mL) were stirred for 10 minutes, followed by the addition of 3,5-dimethylbenzaldehyde (58 mg, 0.43 mmol, 1 eq.). The reaction mixture was stirred at 30 ^oC for 20 hours and the solvent was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], product **190** (75 mg, 55 % yield) was isolated as a yellow solid, **m.p.** 96-98 ^oC. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.77 (1H, br.s, alkene C*H*), 7.69 (1H, dt, *J* = 8.0, 1.0 Hz, ArC*H*), 7.45 (1H, td, *J* = 8.5, 1.0 Hz, ArC*H*), 7.41 (1H, dt, *J* = 8.5, 1.0 Hz, ArC*H*), 7.19 (1H, ddd, *J* = 8.0, 6.5, 1.0 Hz, ArC*H*), 7.09 (2H, s, Ar'C*H*), 7.03 (1H, s, Ar'C*H*), 4.20 (3H, s, NCH_3), 3.24 (2H, td, *J* = 6.0, 1.0 Hz, CH_2CH_2C=C), 3.09 (2H, t, *J* = 6.0 Hz, CH_2CH_2C=C) and 2.40 (6H, s, Ar'CH_3). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ_C 181.9- (*C*=O), 140.4- (*C*), 137.9- (*C*), 137.2- (*C*), 136.3- (*C*), 135.4+ (alkene *C*H), 131.6- (*C*), 129.9+ (Ar'*C*H), 128.3- (*C*), 127.5+ (Ar'*C*H), 126.8+ (Ar*C*H), 124.7- (*C*), 121.3+ (Ar*C*H), 120.1+ (Ar*C*H), 110.3+ (Ar*C*H), 31.7+ (N*C*H_3), 27.7- (CH_2CH_2C=C), 21.4+ (Ar'*C*H_3) and 21.1- (*C*H_2CH_2C=C). **MS** *m/z* (+ESI) 316 (100 %, MH^+) and 338 (15 %, MNa^+). **HRMS** (+ESI) Found MH^+ 316.1701, $C_{22}H_{22}NO$ requires *MH* 316.1701 and found MNa^+ 338.1517, $C_{22}H_{21}NNaO$ requires *MNa* 338.1521. **IR** ν_{max}(liquid film): 3027 (CH), 1651 (C=O) and 1613 (C=C). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^oC) 0.9.

Synthesis of *(E)*-2-(4-hydroxy-3,5-dimethylbenzylidene)-9-methyl-2,3,4,9-tetrahydro-1H-carbazol-1-one 191

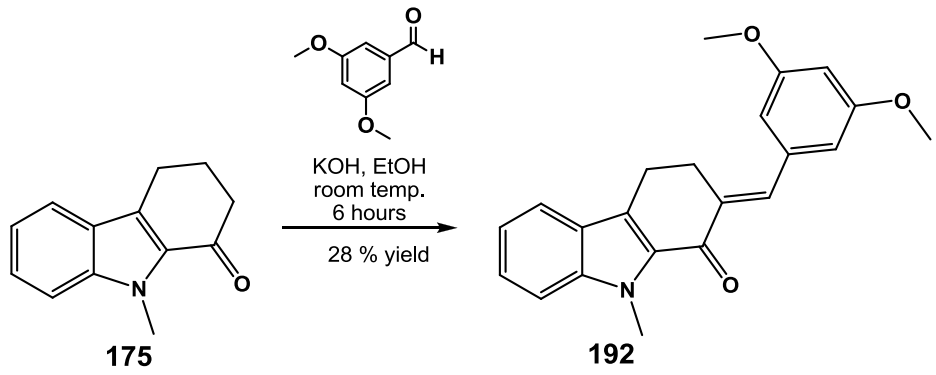


175 191

All glassware was dried under N_2 using a heatgun. Ketone **175** (106 mg, 0.53 mmol, 2 eq.) and 3,5-dimethyl-4-hydroxybenzaldehyde (41 mg, 0.27 mmol, 1 eq.) were added to the flask followed by anhydrous dioxane (2 mL). $BF_3{\cdot}Et_2O$ (0.1 mL, 0.8 mmol, 2.3 eq.) was added slowly and the reaction mixture was heated at 60 ^oC for 14 hours.[160] On cooling, H_2O (40 mL), 2M $NaOH_{(aq.)}$ (20 mL) and EtOAc (40 mL) were added to the reaction mixture. The layers were separated and the aqueous layer was extracted with EtOAc (2 x 40 mL). The combined organic layers were washed with brine (40 mL), dried on Na_2SO_4, filtered and concentrated under reduced pressure to collect 139 mg of crude product. After column chromatography [silica, light petroleum (b.p. 40-60 ^oC) - EtOAc gradient column], product **191** (10 mg, 11 % yield) was isolated as a yellow solid, **m.p.** 203-205 ^oC. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ_H 7.72 (1H, br.s, alkene C*H*), 7.69 (1H, dt, *J* = 8.0, 1.0 Hz, ArC*H*), 7.47-7.43 (1H, m, ArC*H*), 7.41 (1H, dt, *J* = 8.0, 1.0 Hz, ArC*H*), 7.19 (1H, ddd, *J* = 8.0, 6.5, 1.0 Hz, ArC*H*), 7.15 (2H, s, Ar'C*H*), 4.81 (1H, br.s, O*H*), 4.19 (3H, s, NCH_3), 3.26 (2H, td, *J* = 6.5, 1.0 Hz, CH_2CH_2C=CH), 3.09 (2H, t, *J* = 6.5 Hz, CH_2CH_2C=CH) and 2.33 (6H, s, Ar'CH_3). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 181.9- (*C*O), 152.6- (*C*), 140.4- (*C*), 135.5- (*C*), 135.4+ (alkene *C*H), 131.6- (*C*), 130.5+ (Ar'*C*H), 128.5- (*C*), 127.9- (*C*), 126.7+ (Ar*C*H), 124.7- (*C*), 123.0- (*C*), 121.3+ (Ar*C*H), 120.1+ (Ar*C*H), 110.3+ (Ar*C*H), 31.6+ (N*C*H_3), 27.6- (CH_2CH_2C=CH), 21.0- (CH_2CH_2C=CH) and 15.9+ (Ar'*C*H_3). **MS** *m/z* (+ESI) 332 (100 %, MH^+) and 354 (28 %, MNa^+). **HRMS** (+ESI) Found MH^+ 332.1634, $C_{22}H_{22}NO_2$ requires *MH* 332.1651 and found MNa^+ 354.1453, $C_{22}H_{21}NNaO_2$ requires *MNa* 354.1470. **IR** ν_{max}(liquid film): 3608 (OH), 3038 (CH), 1648 (C=O) and 1601 (C=C). **Rf** (30 % EtOAc in light

petroleum (b.p. 40-60 ^{o}C) 0.8. **NOESY NMR** Interaction observed between 7.15 (2H, s, Ar'C*H*) and 3.26 (2H, td, *J* = 6.5, 1.0 Hz, CH_2CH_2C=CH) to confirm *E* configuration.

Synthesis of *(E)*-2-(3,5-dimethoxybenzylidene)-9-methyl-2,3,4,9-tetrahydro-1H-carbazol-1-one 192



175 192

Method A - Optimised method

A mixture of ketone **175** (229 mg, 1.15 mmol, 1 eq.) and 3,5-dimethoxybenzaldehyde (211 mg, 1.27 mmol, 1.1 eq.) were treated with 4 % (w/v) ethanolic KOH (11.5 mL) and stirred at room temperature for 16 hours.[159] The mixture was cooled in an ice-bath and the solid was filtered and washed with EtOH: H_2O (10 mL: 10 mL). H_2O (20 mL) was added to the filtrate which was then neutralised using AcOH, followed by a second filtration. The solid was again washed with EtOH: H_2O (10 mL: 10 mL) to collect **192** (97 mg, **28 % yield**) as a yellow solid.

Method B

Ketone **175** (139 mg, 0.70 mmol, 2 eq.) and $LiOH.H_2O$ (44 mg, 1.05 mmol, 3 eq.) in EtOH (2 mL) were stirred for 10 minutes, followed by the addition of 3,5-dimethoxybenzaldehyde (58 mg, 0.35 mmol, 1 eq.). The reaction mixture was stirred at 30 ^{o}C for 19 hours and the solvent was removed under reduced pressure. After column chromatography [silica, light petroleum (b.p. 40-60 ^{o}C) - EtOAc gradient column], product **192** (31 mg, 25 % yield) was isolated as yellow crystals.

m.p. 134-135 ^{o}C. **^{1}H NMR** (400 MHz; $CDCl_3$): δ_H 7.74 (1H, br.s, alkene C*H*), 7.69 (1H, dt, *J* = 8.0, 1.0 Hz, ArC*H*), 7.46 (1H, ddd, *J* = 8.5, 6.5, 1.0 Hz, ArC*H*), 7.41 (1H, dt, *J* = 8.5, 1.0 Hz, ArC*H*), 7.19 (1H, ddd, *J* = 8.0, 6.5, 1.0 Hz, ArC*H*), 6.61 (2H, dd, *J* = 2.5, 0.5 Hz, Ar'C*H*), 6.50 (1H, t, *J* = 2.5 Hz, Ar'C*H*), 4.19 (3H, s, NCH_3), 3.86 (6H, s, Ar'OCH_3), 3.24

(2H, td, *J* = 6.5, 1.0 Hz, CH_2CH_2C=CH), 3.09 (2H, t, *J* = 6.5 Hz, CH_2CH_2C=CH). **^{13}C NMR** (100 MHz; $CDCl_3$): δ_C 181.7- (*C*=O), 160.7- (*C*), 140.5- (*C*), 138.3- (*C*), 138.0- (*C*), 134.9+ (alkene *C*H), 131.5- (*C*), 128.6- (*C*), 126.9+ (Ar*C*H), 124.6- (*C*), 121.4+ (Ar*C*H), 120.2+ (Ar*C*H), 110.4+ (Ar*C*H), 107.7+ (Ar'*C*H), 100.2+ (Ar'*C*H), 55.4+ (Ar'OCH_3), 31.7+ (NCH_3), 27.7- (CH_2CH_2C=CH) and 21.1 (CH_2CH_2C=CH). **MS** *m/z* (+ESI) 348 (100 %, MH^+) and 370 (18 %, MNa^+) **HRMS** (+ESI) Found MH^+ 348.1606, $C_{22}H_{22}NO_3$ requires *MH* 348.1600 and found MNa^+ 370.1423, $C_{22}H_{21}NNaO_3$ requires *MNa* 370.1419. **IR** ν_{max}(liquid film): 3026 (CH), 1652 (C=O) and 1594 (C=C). **Rf** (30 % EtOAc in light petroleum (b.p. 40-60 ^oC) 0.8. **Analysis** (Found: H, 6.06; N, 3.93. $C_{22}H_{21}NO_3$ requires H, 6.09; N, 4.03 %).

8. Appendices

8.1. Chapter Two

8.1.1. CO_2 Labelling experiments

NMR

Simultaneous equations: **x** is the proportion of the integral corresponding to the ^{12}C compound and **y** is the proportion of the integral corresponding to the ^{13}C compound.

Owing to different relaxation times, the integral of the C=O peak differs to the integrals of the remaining peaks. From the background ^{13}C NMR spectra, we can determine the difference in the integrals, which is incorporated in the equation as the "integration factor".

In addition, the C=O signal for the ^{13}C compound **x** will be 100 % abundant, so in order to make a direct comparison with the C=O signal for the ^{12}C compound **y**, we must use a conversion factor which take into account the natural abundance of ^{13}C which is 1.11 %. For all other C signals, **x** + **y** will simply equal the integral of that peak.

Measured ratio 0:100 $NaH^{12}CO_3$:$NaH^{13}CO_3$

Comparison of C=O peak at 155.8 ppm integral 23.9895 with 68.2 ppm integral 1.0000
Integration factor = 3.6904

1) x + y = 1.0000
2) x + (100y/1.11) = 23.9895 × 3.6904

Rearranging Eq. 1 to y = 1.0000 – x and substituting into Eq. 2 gives:

3) x + (100(1 – x)/1.11) = 88.5309, becomes
4) 90.0901 – 89.0901x = 88.5309, becomes
5) 89.0901x = 1.5592,
6) x = 0.0175

From Eq. 1, x + y = 1.0000; x = 0.0175 and y = 0.9825, ^{12}C = 1.75% and ^{13}C = 98.25%

Comparison of C=O peak at 155.8 ppm integral 23.9895 with 54.5 ppm integral 0.7221

Integration factor = 2.6110

1) $x + y = 0.7221$
2) $x + (100y/1.11) = 23.9895 \times 2.6110$

Rearranging Eq. 1 to $y = 0.7221 – x$ and substituting into Eq. 2 gives:

3) $x + (100(0.7221 – x)/1.11) = 62.6366$, becomes
4) $66.0541 – 89.0901x = 62.6366$, becomes
5) $89.0901x = 2.4175$,
6) $x = 0.0271$

From Eq. 1, $x + y = 0.7221$, $x = 0.0271$ and $y = 0.6950$, $^{12}C = 3.8\%$ and $^{13}C = 96.2\%$

Comparison of C=O peak at 155.8 ppm integral 23.9895 with 31.3 ppm integral 0.9621
Integration factor = 3.5564

1) $x + y = 0.9621$
2) $x + (100y/1.11) = 23.9895 \times 3.5564$

Rearranging Eq. 1 to $y = 0.9621 – x$ and substituting into Eq. 2 gives:

3) $x + (100(0.9621 – x)/1.11) = 85.3163$, becomes
4) $86.6757 – 89.0901x = 66.2632$, becomes
5) $89.0901x = 1.3594$,
6) $x = 0.0153$

From Eq. 1, $x + y = 0.9621$; $x = 0.0153$ and $y = 0.9468$, $^{12}C = 1.6\%$ and $^{13}C = 98.4\%$

Comparison of C=O peak at 155.8 ppm integral 23.9895 with 28.6 ppm integral 0.9770
Integration factor = 3.5026

1) $x + y = 0.9770$
2) $x + (100y/1.11) = 23.9895 \times 3.5026$

Rearranging Eq. 1 to $y = 0.9770 – x$ and substituting into Eq. 2 gives:

3) $x + (100(0.9770 – x)/1.11) = 84.0256$, becomes
4) $88.0180 – 89.0901x = 84.0256$, becomes
5) $89.0901x = 3.9924$,
6) $x = 0.0448$

From Eq. 1, $x + y = 0.9770$; $x = 0.0448$ and $y = 0.9322$, $^{12}C = 4.6\%$ and $^{13}C = 95.4\%$

Comparison of C=O peak at 155.8 ppm integral 23.9895 with 25.3 ppm integral 0.9535

Integration factor = 3.4658

1) $x + y = 0.9535$
2) $x + (100y/1.11) = 23.9895 \times 3.4658$

Rearranging Eq. 1 to $y = 0.9535 – x$ and substituting into Eq. 2 gives:

3) $x + (100(0.9535 – x)/1.11) = 83.1428$, becomes
4) $85.9009 – 89.0901x = 83.1428$, becomes
5) $89.0901x = 2.7581$,
6) $x = 0.0310$

From Eq. 1, $x + y = 0.9535$; $x = 0.0310$ and $y = 0.9225$, $^{12}C = 3.2\%$ and $^{13}C = 96.8\%$

Comparison of C=O peak at 155.8 ppm integral 23.9895 with 22.4 ppm integral 0.9107
Integration factor = 3.3807

1) $x + y = 0.9107$
2) $x + (100y/1.11) = 23.9895 \times 3.3807$

Rearranging Eq. 1 to $y = 0.9107 – x$ and substituting into Eq. 2 gives:

3) $x + (100(0.9107 – x)/1.11) = 81.1013$, becomes
4) $82.0450 – 89.0901x = 81.1013$, becomes
5) $89.0901x = 0.9437$,
6) $x = 0.0106$

From Eq. 1, $x + y = 0.9107$; $x = 0.0106$ and $y = 0.9001$, $^{12}C = 1.2\%$ and $^{13}C = 98.8\%$

Comparison of C=O peak at 155.8 ppm integral 23.9895 with 13.9 ppm integral 0.9068
Integration factor = 3.4515

1) $x + y = 0.9068$
2) $x + (100y/1.11) = 23.9895 \times 3.4515$

Rearranging Eq. 1 to $y = 0.9068 – x$ and substituting into Eq. 2 gives:

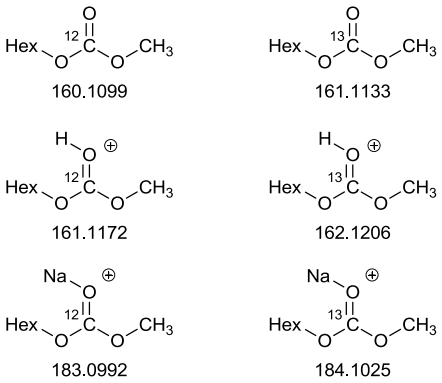
3) $x + (100(0.9068 – x)/1.11) = 82.7998$, becomes
4) $81.6937 – 89.0901x = 82.7998$, becomes
5) $89.0901x = –1.1061$,
6) $x = –0.0124$

From Eq. 1, $x + y = 0.9068$; $x = –0.0124$ and $y = 0.9192$, $^{12}C = –1.4\%$ and $^{13}C = 101.4\%$

Average of the 7 peaks

$$\frac{1.75 + 3.8 + 1.6 + 4.6 + 3.2 + 1.2 - 1.4}{7} = \underline{\underline{\mathbf{^{12}C = 2.1\% \text{ and } ^{13}C = 97.9\%}}}$$

Determination of ratios by High Resolution Mass spectrometry



x **y**

m/z of 100 % ^{12}C compound **x** shows the MNa^+ ion at 183.1035 (39 090, 91.26 %) and 184.1067 (3 746, 8.74 %), due to natural abundance of ^{13}C present in this compound. Using this background reading, the ratios of the mixtures are calculated below.

The proportion of the peak at 184.1 due to the natural abundance of ^{13}C is (3 746/39 090) of the peak at 183.09.

x is the proportion of the integral corresponding to the ^{12}C compound and **y** is the proportion of the integral corresponding to the ^{13}C compound.

Measured ratio 0:100 $NaH^{12}CO_3$:$NaH^{13}CO_3$

MNa^+ 183.0973, integration 24 203 (is solely due to compound x)
MNa^+ 184.1008, integration 800 762 (is due to the natural abundance of ^{13}C in compound x plus compound y), therefore

1) x = 24 203

2) (3 746/39 090)x + y = 800 762

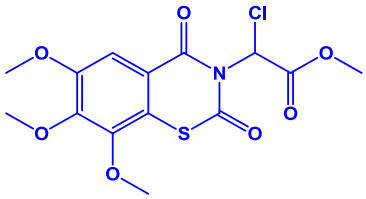
Substituting Eq. 1 into Eq. 2 gives:

3) (3 746/39 090) × 24 203 + y = 800 762, becomes

4) y = 798 442.623

From Eq. 1 x = 24 203 and Eq. 4 y = 798 442.623, **^{12}C = 2.9% and ^{13}C = 97.1%**

8.1.2. Crystal Structure Data - Compound 69



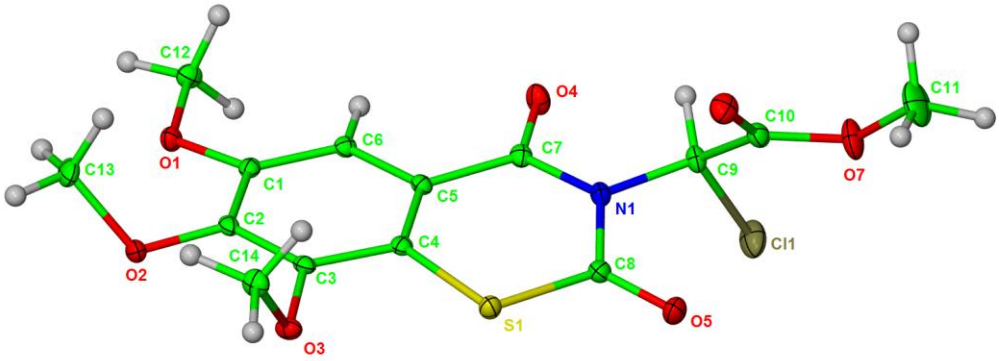


Table 1. Crystal data and structure refinement for compound **69**

Identification code	h10farm1
Empirical formula	C14 H14 Cl N O7 S
Formula weight	375.77
Temperature	150(2) K

Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P21/a
Unit cell dimensions	a = 7.2820(1)Å $\alpha = 90^o$
	b = 18.8380(4)Å $\beta = 102.451(1)^o$
	c = 11.8360(3)Å $\gamma = 90^o$
Volume	1585.46(6) $Å^3$
Z	4
Density (calculated)	1.574 Mg/m^3
Absorption coefficient	0.410 mm^{-1}
F(000)	776
Crystal size	0.30 x 0.15 x 0.15 mm
Theta range for data collection	2.07 to 27.48^o
Index ranges	-9<=h<=9; -23<=k<=24; -15<=l<=15
Reflections collected	17860
Independent reflections	3624 [R(int) = 0.0402]
Reflections observed (>2σ)	2843
Data Completeness	0.997
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.938 and 0.894
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3624 / 0 / 222
Goodness-of-fit on F^2	1.050
Final R indices [I>2σ(I)]	R1 = 0.0361 wR2 = 0.0837
R indices (all data)	R1 = 0.0550 wR2 = 0.0933
Largest diff. peak and hole	0.301 and -0.362 $eÅ^{-3}$

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$ x 10^3) for compound **69**.U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)
Cl(1)	3558(1)	1580(1)	5842(1)	43(1)
S(1)	3991(1)	929(1)	9922(1)	24(1)
O(1)	3892(2)	3842(1)	11704(1)	24(1)
O(2)	3433(2)	2686(1)	13007(1)	25(1)
O(3)	3467(2)	1342(1)	12107(1)	25(1)
O(4)	5331(2)	2818(1)	8029(1)	30(1)
O(5)	4092(2)	470(1)	7900(1)	35(1)
O(6)	7994(2)	770(1)	7852(1)	35(1)
O(7)	6896(2)	758(1)	5932(1)	45(1)
N(1)	5002(2)	1623(1)	8120(1)	22(1)
C(1)	4086(2)	3192(1)	11243(2)	20(1)
C(2)	3840(2)	2608(1)	11938(1)	20(1)
C(3)	3864(2)	1926(1)	11507(2)	20(1)
C(4)	4169(2)	1813(1)	10391(2)	19(1)
C(5)	4504(2)	2388(1)	9730(2)	19(1)
C(6)	4444(2)	3080(1)	10156(2)	20(1)
C(7)	4959(2)	2315(1)	8584(2)	21(1)
C(8)	4340(3)	992(1)	8509(2)	24(1)

C(9)	5559(3)	1583(1)	7020(2)	25(1)
C(10)	6933(3)	973(1)	7005(2)	27(1)
C(11)	8353(4)	251(2)	5829(2)	61(1)
C(12)	4225(3)	4450(1)	11046(2)	27(1)
C(13)	4759(3)	3083(1)	13850(2)	33(1)
C(14)	4986(3)	1122(1)	13040(2)	29(1)

Table 3. Bond lengths [Å] and angles [°] for compound **69**.

Cl(1)-C(9)	1.7864(19)	S(1)-C(8)	1.7487(19)
S(1)-C(4)	1.7509(17)	O(1)-C(1)	1.361(2)
O(1)-C(12)	1.434(2)	O(2)-C(2)	1.368(2)
O(2)-C(13)	1.441(2)	O(3)-C(3)	1.375(2)
O(3)-C(14)	1.445(2)	O(4)-C(7)	1.215(2)
O(5)-C(8)	1.208(2)	O(6)-C(10)	1.189(2)
O(7)-C(10)	1.328(2)	O(7)-C(11)	1.452(3)
N(1)-C(8)	1.399(2)	N(1)-C(7)	1.416(2)
N(1)-C(9)	1.447(2)	C(1)-C(6)	1.383(2)
C(1)-C(2)	1.408(2)	C(2)-C(3)	1.384(3)
C(3)-C(4)	1.401(2)	C(4)-C(5)	1.389(2)
C(5)-C(6)	1.402(2)	C(5)-C(7)	1.471(2)
C(6)-H(6)	0.9500	C(9)-C(10)	1.526(3)
C(9)-H(9)	1.0000	C(11)-H(11A)	0.9800
C(11)-H(11B)	0.9800	C(11)-H(11C)	0.9800
C(12)-H(12A)	0.9800	C(12)-H(12B)	0.9800
C(12)-H(12C)	0.9800	C(13)-H(13A)	0.9800
C(13)-H(13B)	0.9800	C(13)-H(13C)	0.9800
C(14)-H(14A)	0.9800	C(14)-H(14B)	0.9800
C(14)-H(14C)	0.9800		
C(8)-S(1)-C(4)	102.93(9)	C(1)-O(1)-C(12)	117.18(14)
C(2)-O(2)-C(13)	116.68(14)	C(3)-O(3)-C(14)	114.35(13)
C(10)-O(7)-C(11)	114.58(18)	C(8)-N(1)-C(7)	128.10(15)
C(8)-N(1)-C(9)	115.72(15)	C(7)-N(1)-C(9)	115.56(14)
O(1)-C(1)-C(6)	124.50(16)	O(1)-C(1)-C(2)	115.58(15)
C(6)-C(1)-C(2)	119.88(16)	O(2)-C(2)-C(3)	117.57(15)
O(2)-C(2)-C(1)	122.50(16)	C(3)-C(2)-C(1)	119.74(15)
O(3)-C(3)-C(2)	122.11(15)	O(3)-C(3)-C(4)	117.34(16)
C(2)-C(3)-C(4)	120.39(16)	C(5)-C(4)-C(3)	119.68(16)
C(5)-C(4)-S(1)	124.96(13)	C(3)-C(4)-S(1)	115.30(13)
C(4)-C(5)-C(6)	120.01(16)	C(4)-C(5)-C(7)	123.26(16)
C(6)-C(5)-C(7)	116.72(15)	C(1)-C(6)-C(5)	120.16(16)
C(1)-C(6)-H(6)	119.9	C(5)-C(6)-H(6)	119.9
O(4)-C(7)-N(1)	118.76(16)	O(4)-C(7)-C(5)	123.07(16)
N(1)-C(7)-C(5)	118.16(15)	O(5)-C(8)-N(1)	120.90(17)
O(5)-C(8)-S(1)	118.80(15)	N(1)-C(8)-S(1)	120.19(13)
N(1)-C(9)-C(10)	111.25(15)	N(1)-C(9)-Cl(1)	111.30(13)
C(10)-C(9)-Cl(1)	114.51(13)	N(1)-C(9)-H(9)	106.4
C(10)-C(9)-H(9)	106.4	Cl(1)-C(9)-H(9)	106.4
O(6)-C(10)-O(7)	125.73(18)	O(6)-C(10)-C(9)	122.70(17)

O(7)-C(10)-C(9)	111.27(16)	O(7)-C(11)-H(11A)	109.5
O(7)-C(11)-H(11B)	109.5	H(11A)-C(11)-H(11B)	109.5
O(7)-C(11)-H(11C)	109.5	H(11A)-C(11)-H(11C)	109.5
H(11B)-C(11)-H(11C)	109.5	O(1)-C(12)-H(12A)	109.5
O(1)-C(12)-H(12B)	109.5	H(12A)-C(12)-H(12B)	109.5
O(1)-C(12)-H(12C)	109.5	H(12A)-C(12)-H(12C)	109.5
H(12B)-C(12)-H(12C)	109.5	O(2)-C(13)-H(13A)	109.5
O(2)-C(13)-H(13B)	109.5	H(13A)-C(13)-H(13B)	109.5
O(2)-C(13)-H(13C)	109.5	H(13A)-C(13)-H(13C)	109.5
H(13B)-C(13)-H(13C)	109.5	O(3)-C(14)-H(14A)	109.5
O(3)-C(14)-H(14B)	109.5	H(14A)-C(14)-H(14B)	109.5
O(3)-C(14)-H(14C)	109.5	H(14A)-C(14)-H(14C)	109.5
H(14B)-C(14)-H(14C)	109.5		

Table 4. Anisotropic displacement parameters ($Å^2$ x 10^3) for compound **69**. The anisotropic displacement factor exponent takes the form: -2 gpi^2 [h^2 a^{*2} U11 + ... + 2 h k a* b* U

Atom	U11	U22	U33	U23	U13	U12
Cl(1)	40(1)	62(1)	23(1)	-4(1)	-1(1)	14(1)
S(1)	29(1)	20(1)	25(1)	-2(1)	9(1)	-3(1)
O(1)	28(1)	20(1)	27(1)	-3(1)	10(1)	1(1)
O(2)	25(1)	30(1)	20(1)	-4(1)	8(1)	-4(1)
O(3)	24(1)	25(1)	25(1)	4(1)	6(1)	-4(1)
O(4)	44(1)	24(1)	27(1)	4(1)	16(1)	4(1)
O(5)	43(1)	30(1)	35(1)	-11(1)	15(1)	-9(1)
O(6)	33(1)	33(1)	37(1)	-1(1)	5(1)	8(1)
O(7)	53(1)	54(1)	33(1)	-8(1)	19(1)	19(1)
N(1)	23(1)	24(1)	19(1)	-1(1)	7(1)	2(1)
C(1)	15(1)	21(1)	24(1)	-4(1)	3(1)	1(1)
C(2)	15(1)	27(1)	19(1)	-2(1)	4(1)	0(1)
C(3)	15(1)	24(1)	21(1)	1(1)	4(1)	-2(1)
C(4)	14(1)	21(1)	22(1)	-2(1)	3(1)	0(1)
C(5)	15(1)	22(1)	20(1)	0(1)	4(1)	1(1)
C(6)	17(1)	20(1)	23(1)	0(1)	5(1)	2(1)
C(7)	20(1)	23(1)	22(1)	0(1)	4(1)	3(1)
C(8)	23(1)	25(1)	26(1)	-4(1)	7(1)	-3(1)
C(9)	27(1)	28(1)	19(1)	-1(1)	7(1)	4(1)
C(10)	28(1)	26(1)	29(1)	-4(1)	12(1)	0(1)
C(11)	65(2)	64(2)	64(2)	-14(1)	34(1)	26(1)
C(12)	28(1)	19(1)	34(1)	0(1)	9(1)	1(1)
C(13)	31(1)	45(1)	21(1)	-10(1)	3(1)	-3(1)
C(14)	32(1)	30(1)	24(1)	7(1)	7(1)	1(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for compound **69**.

Atom	x	y	z	U(eq)
H(6)	4649	3474	9696	24
H(9)	6264	2030	6947	29

H(11A)	9586	482	6036	92
H(11B)	8142	78	5031	92
H(11C)	8315	-150	6352	92
H(12A)	5525	4440	10944	40
H(12B)	4017	4883	11458	40
H(12C)	3360	4441	10287	40
H(13A)	6033	3005	13727	49
H(13B)	4693	2926	14629	49
H(13C)	4455	3590	13766	49
H(14A)	6124	1045	12739	43
H(14B)	4641	679	13377	43
H(14C)	5225	1492	13635	43

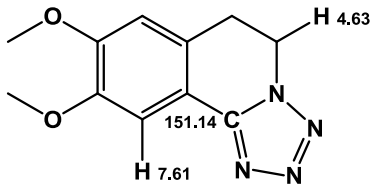
Table 6. Dihedral angles [^{o}] for compound **69**.

Atom1 - Atom2 - Atom3 - Atom4	Dihedral
C(12) - O(1) - C(1) - C(6)	-4.9(2)
C(12) - O(1) - C(1) - C(2)	176.97(15)
C(13) - O(2) - C(2) - C(3)	126.48(17)
C(13) - O(2) - C(2) - C(1)	-58.5(2)
O(1) - C(1) - C(2) - O(2)	0.0(2)
C(6) - C(1) - C(2) - O(2)	-178.16(14)
O(1) - C(1) - C(2) - C(3)	174.96(14)
C(6) - C(1) - C(2) - C(3)	-3.2(2)
C(14) - O(3) - C(3) - C(2)	-77.5(2)
C(14) - O(3) - C(3) - C(4)	106.94(17)
O(2) - C(2) - C(3) - O(3)	1.1(2)
C(1) - C(2) - C(3) - O(3)	-174.11(15)
O(2) - C(2) - C(3) - C(4)	176.46(14)
C(1) - C(2) - C(3) - C(4)	1.3(2)
O(3) - C(3) - C(4) - C(5)	177.52(14)
C(2) - C(3) - C(4) - C(5)	1.9(2)
O(3) - C(3) - C(4) - S(1)	0.10(19)
C(2) - C(3) - C(4) - S(1)	-175.52(13)
C(8) - S(1) - C(4) - C(5)	0.19(17)
C(8) - S(1) - C(4) - C(3)	177.46(13)
C(3) - C(4) - C(5) - C(6)	-3.2(2)
S(1) - C(4) - C(5) - C(6)	174.00(13)
C(3) - C(4) - C(5) - C(7)	175.92(15)
S(1) - C(4) - C(5) - C(7)	-6.9(2)
O(1) - C(1) - C(6) - C(5)	-176.04(15)
C(2) - C(1) - C(6) - C(5)	2.0(2)
C(4) - C(5) - C(6) - C(1)	1.2(2)
C(7) - C(5) - C(6) - C(1)	-177.92(14)
C(8) - N(1) - C(7) - O(4)	-168.67(17)
C(9) - N(1) - C(7) - O(4)	1.9(2)
C(8) - N(1) - C(7) - C(5)	12.7(3)
C(9) - N(1) - C(7) - C(5)	-176.71(14)
C(4) - C(5) - C(7) - O(4)	-176.70(17)

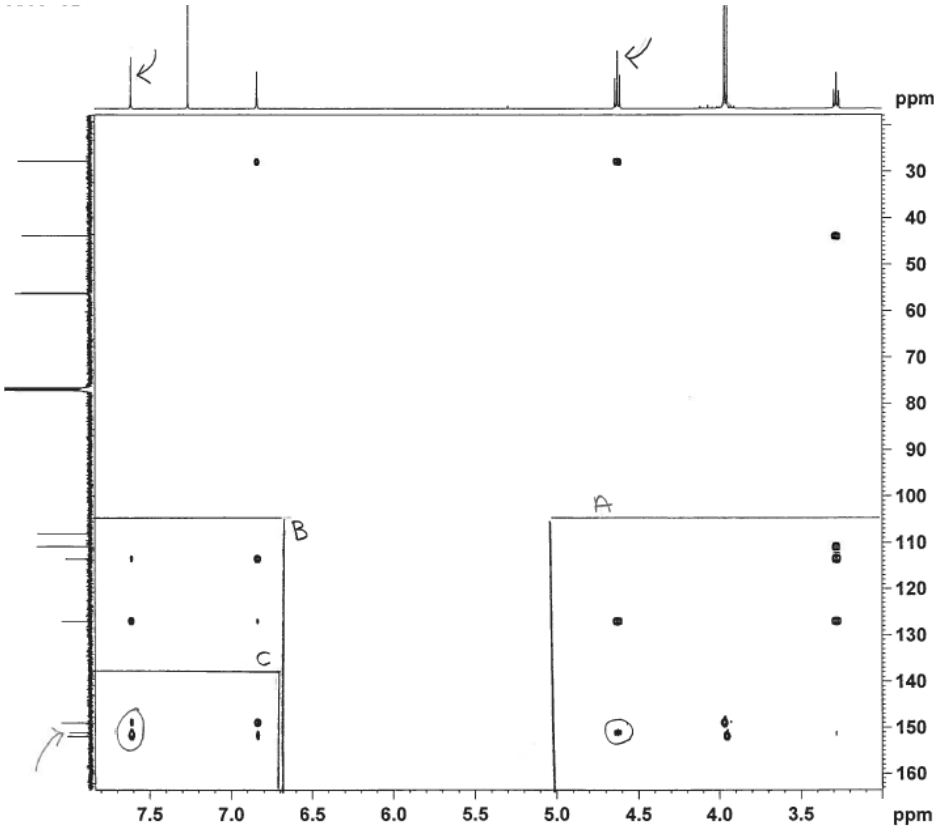
C(6) - C(5) - C(7) - O(4)	2.4(2)
C(4) - C(5) - C(7) - N(1)	1.9(2)
C(6) - C(5) - C(7) - N(1)	-179.03(15)
C(7) - N(1) - C(8) - O(5)	163.85(17)
C(9) - N(1) - C(8) - O(5)	-6.7(3)
C(7) - N(1) - C(8) - S(1)	-20.0(2)
C(9) - N(1) - C(8) - S(1)	169.41(13)
C(4) - S(1) - C(8) - O(5)	-171.89(15)
C(4) - S(1) - C(8) - N(1)	11.89(16)
C(8) - N(1) - C(9) - C(10)	-51.7(2)
C(7) - N(1) - C(9) - C(10)	136.52(16)
C(8) - N(1) - C(9) - Cl(1)	77.30(17)
C(7) - N(1) - C(9) - Cl(1)	-94.48(16)
C(11) - O(7) - C(10) - O(6)	-2.4(3)
C(11) - O(7) - C(10) - C(9)	171.47(19)
N(1) - C(9) - C(10) - O(6)	-29.8(3)
Cl(1) - C(9) - C(10) - O(6)	-157.10(16)
N(1) - C(9) - C(10) - O(7)	156.09(16)
Cl(1) - C(9) - C(10) - O(7)	28.8(2)

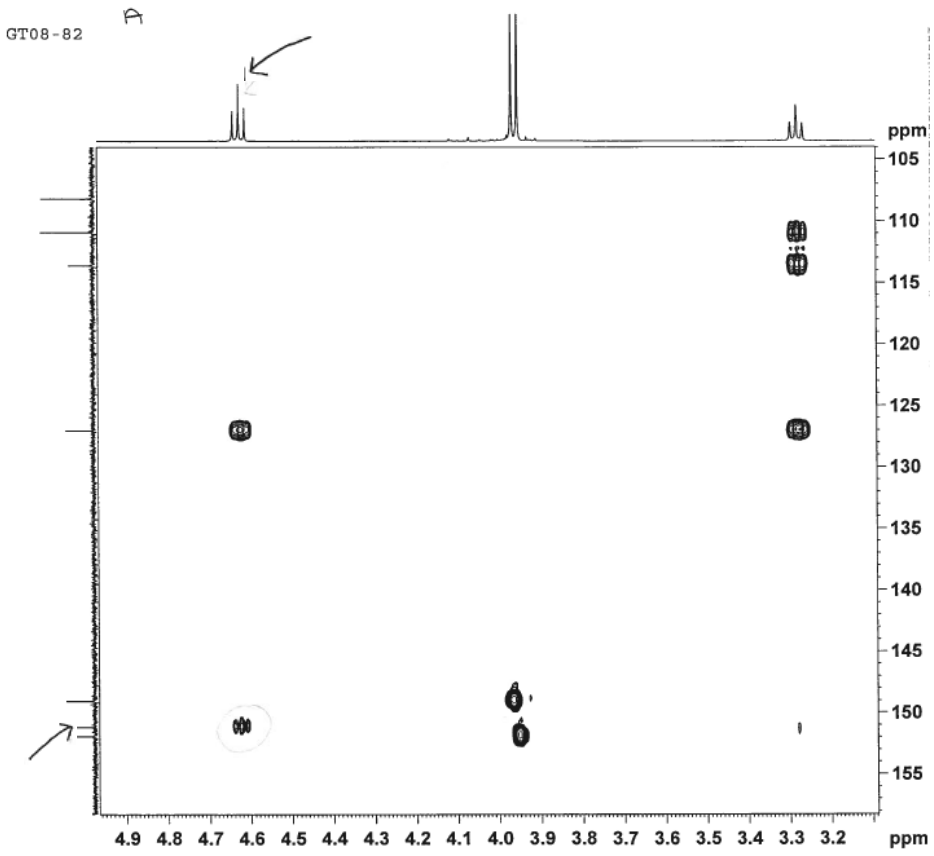
8.2. Chapter Three

8.2.1. HMBC of Tetrazole 120



Imine carbon at 151.14 ppm can be seen from not only the proton at 4.63 ppm but also from the proton at 7.61 ppm. This would only occur if the tetrazole was as the regioisomer shown. There is an overlap of the interaction between imine carbon 151.14 ppm and the proton at 7.61 ppm in the HMBC but it can clearly be seen (see Expansion C).




GT08-82
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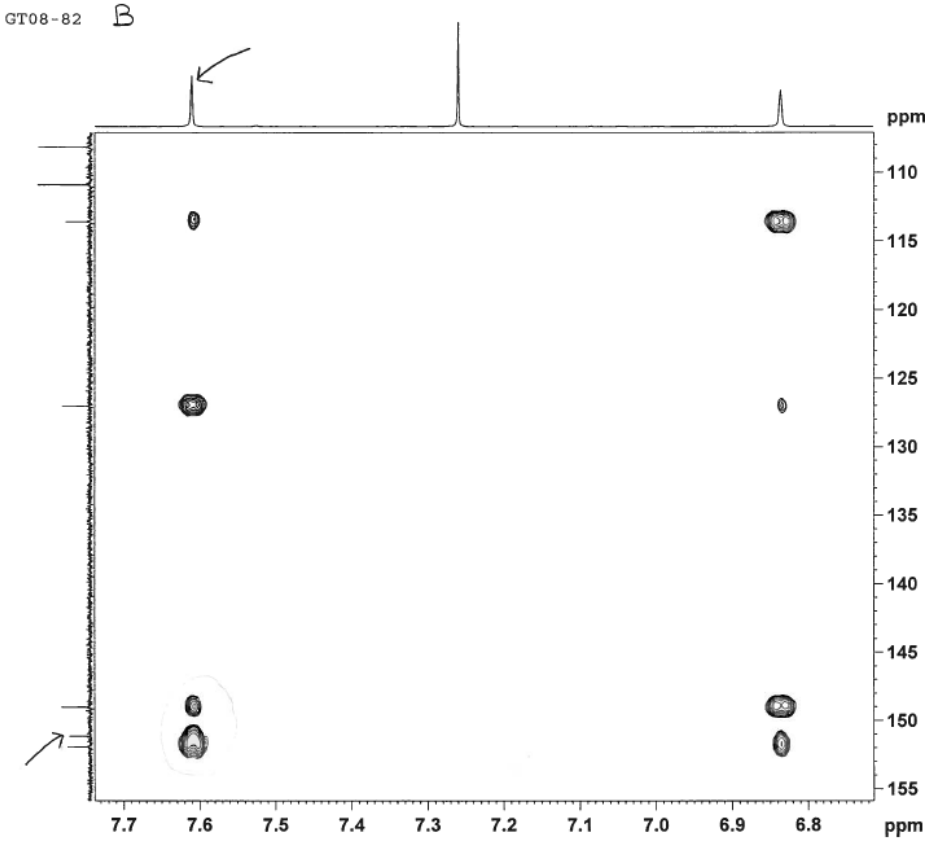
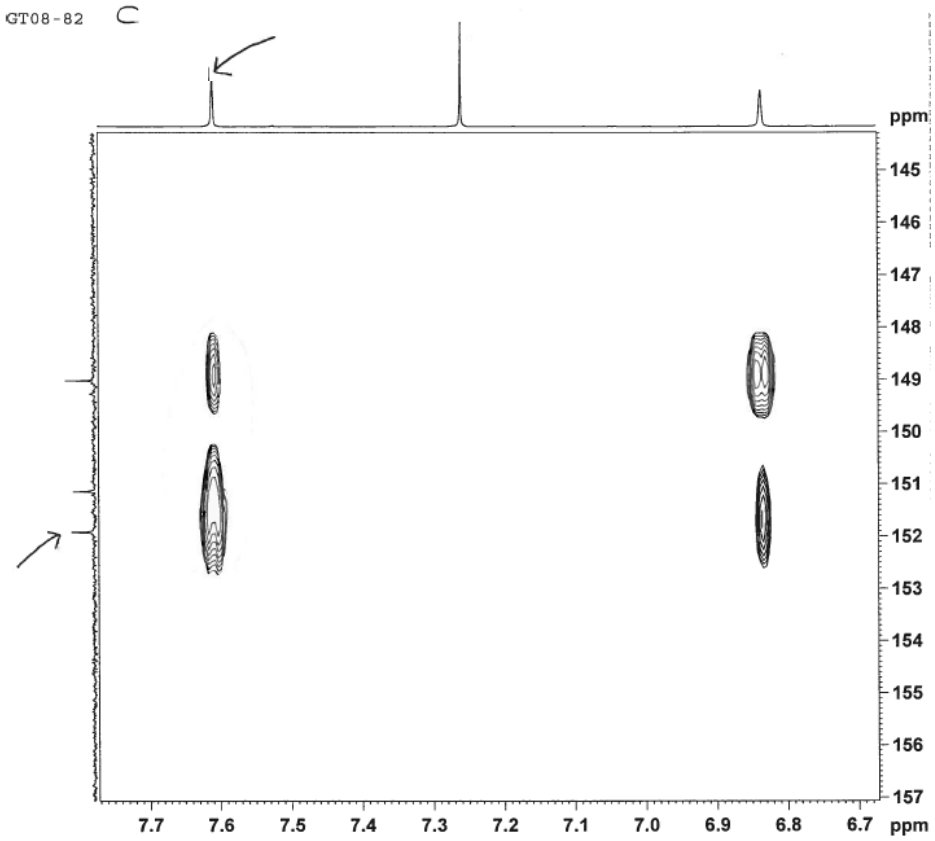
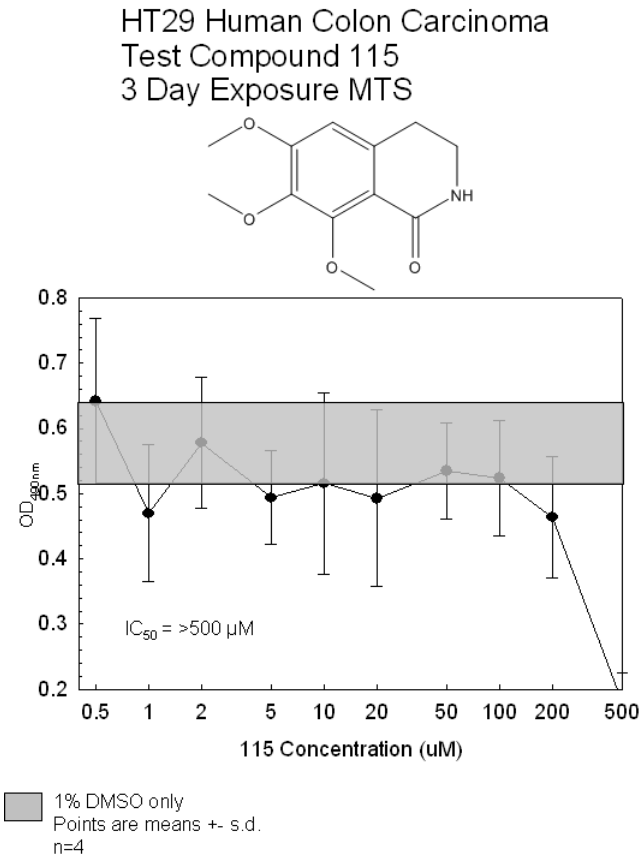
ppm
105
110
115
120
125
130
135
140
145
150
155
4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2
ppm

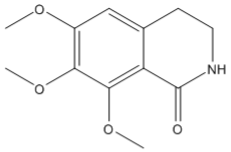

GT08-82
B
ppm
110
115
120
125
130
135
140
145
150
155
7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8
ppm

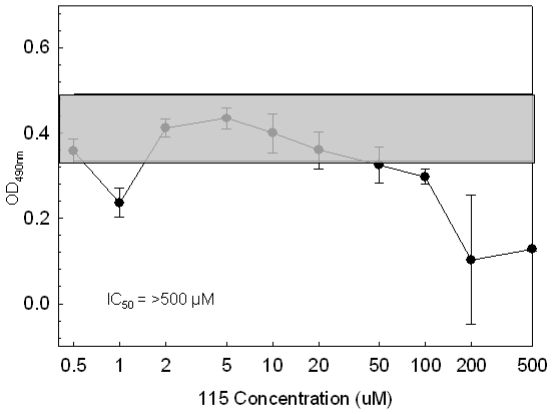

GT08-82
ppm
145
146
147
148
149
150
151
152
153
154
155
156
157
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
6.8
6.7
ppm

8.2.2. IC_{50} Values of A/B Lactams and Tetrazoles in HT29 and MDA231 Cancer Cell Lines



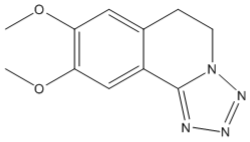
MDA231 Breast Cell Line
Test Compound 115
3 Day Exposure MTS

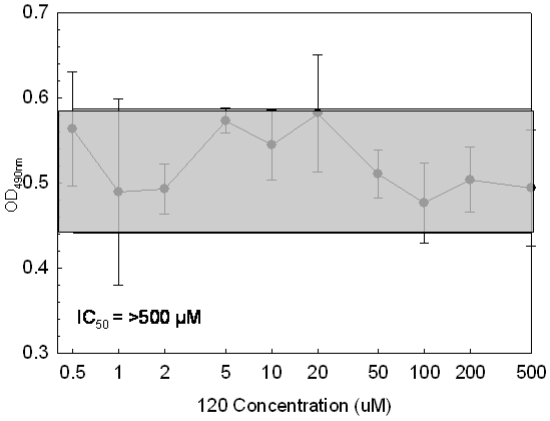




1% DMSO only
Points are means +- s.d.
n=4

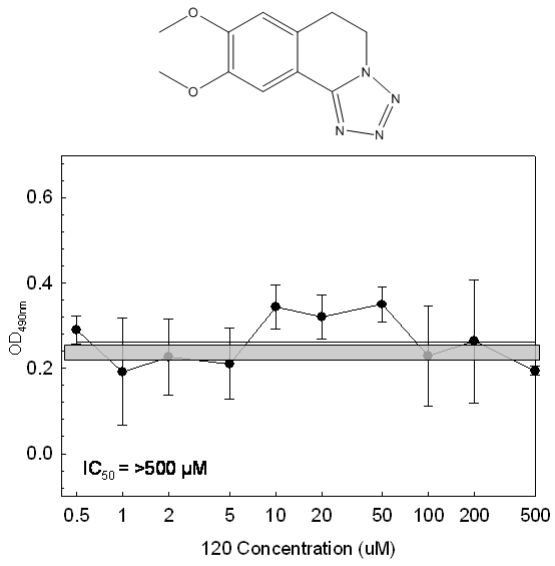
HT29 Human Colon Carcinoma
Test Compound 120
3 Day Exposure MTS





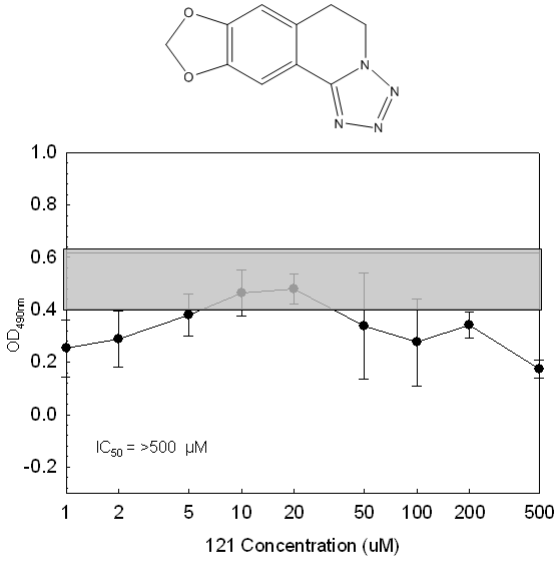
1% DMSO only
Points are means +- s.d.
n=4

MDA231 Breast Cell Line
Test Compound 120
3 Day Exposure MTS



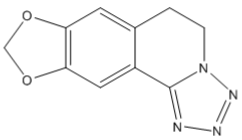
1% DMSO only
Points are means +- s.d.
n=4

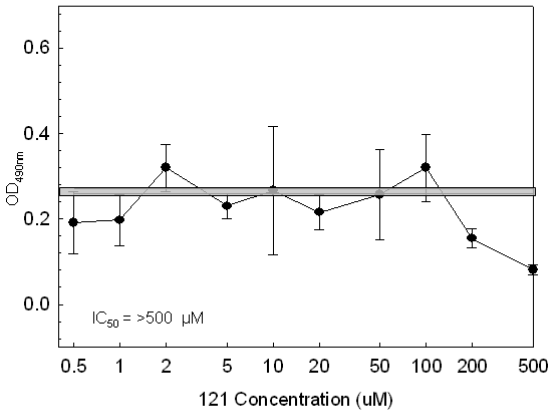
HT29 Human Colon Carcinoma
Test Compound 121
3 Day Exposure MTS



1% DMSO only
Points are means +- s.d.
n=4

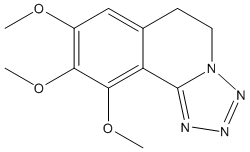
MDA231 Breast Cell Line
Test Compound 121
3 Day Exposure MTS

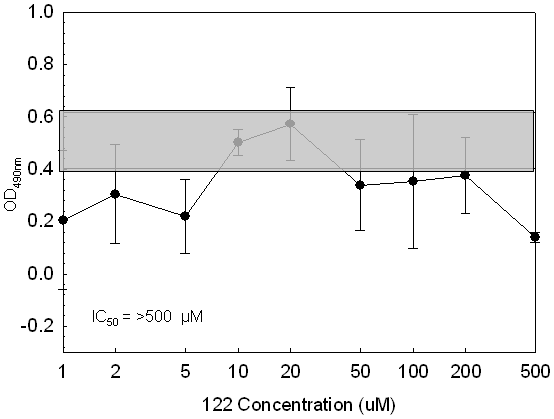




1% DMSO only
Points are means +- s.d.
n=4

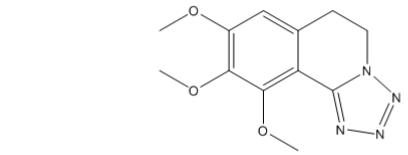
HT29 Human Colon Carcinoma
Test Compound 122
3 Day Exposure MTS

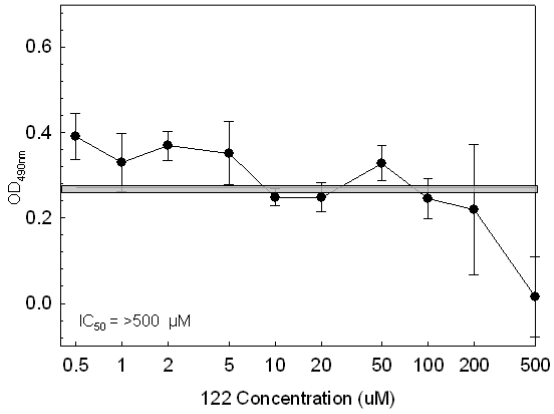




1% DMSO only
Points are means +- s.d.
n=4

MDA231 Breast Cell Line
Test Compound 122
3 Day Exposure MTS

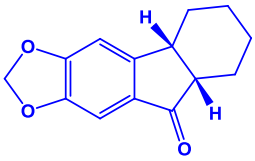




1% DMSO only
Points are means +- s.d.
n=4

8.3. Chapter Four

8.3.1. Crystal Structure Data - Indanone 136



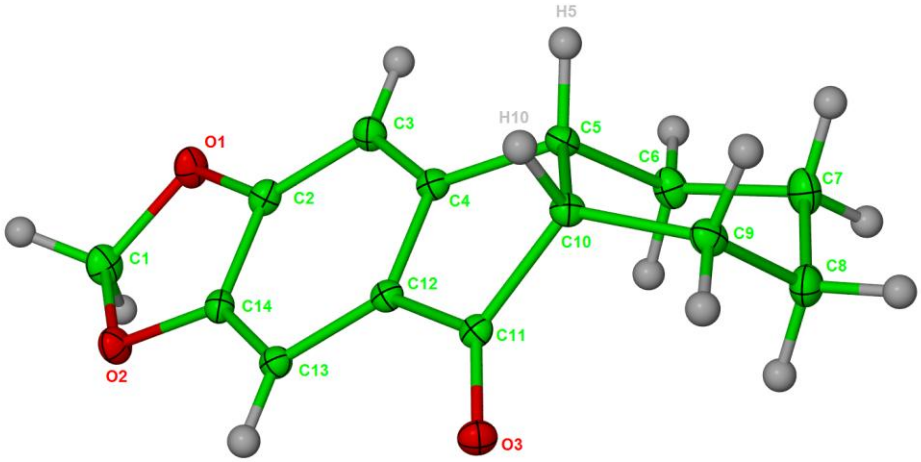


Table 1. Crystal data and structure refinement for compound **136**.

Identification code	p09farm1
Empirical formula	C14 H14 O3
Formula weight	230.25
Temperature	150(2) K
Wavelength	1.54184 Å
Crystal system	Monoclinic
Space group	P21/n
Unit cell dimensions	a = 10.1099(3)Å $\alpha = 90^{o}$
	b = 5.0318(2)Å $\beta = 91.870(3)^{o}$
	c = 21.5241(6)Å $\gamma = 90^{o}$
Volume	1094.37(6) $Å^3$
Z	4
Density (calculated)	1.397 Mg/m^3
Absorption coefficient	0.797 mm^{-1}
F(000)	488
Crystal size	0.18 x 0.15 x 0.08 mm

Theta range for data collection	7.69 to 62.30 ^{o}.
Index ranges	-11<=h<=11; -5<=k<=5; -24<=l<=24
Reflections collected	34832
Independent reflections	1726 [R(int) = 0.0286]
Reflections observed (>2σ)	1579
Data Completeness	0.997
Max. and min. transmission	1.0000, 0.4681
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1726 / 0 / 155
Goodness-of-fit on F^2	1.079
Final R indices [I>2σ(I)]	R1 = 0.0292 wR2 = 0.0734
R indices (all data)	R1 = 0.0320 wR2 = 0.0754
Largest diff. peak and hole	0.185 and -0.137 eÅ^{-3}

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3) for compound **136**.U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

Atom	x	y	z	U(eq)
O(1)	6861(1)	4915(2)	1454(1)	33(1)
O(2)	7812(1)	1947(2)	2159(1)	31(1)
O(3)	13128(1)	4300(2)	2010(1)	27(1)
C(1)	6604(1)	2643(3)	1838(1)	35(1)
C(2)	8205(1)	5255(3)	1480(1)	25(1)
C(3)	8922(1)	7063(3)	1153(1)	27(1)
C(4)	10290(1)	7028(3)	1278(1)	23(1)
C(5)	11342(1)	8592(3)	955(1)	25(1)
C(6)	11591(1)	7374(3)	313(1)	31(1)
C(7)	12926(1)	8168(3)	62(1)	37(1)
C(8)	14035(1)	7258(3)	509(1)	35(1)
C(9)	13917(1)	8598(3)	1137(1)	29(1)
C(10)	12554(1)	8292(3)	1409(1)	24(1)
C(11)	12295(1)	5702(3)	1747(1)	22(1)
C(12)	10853(1)	5265(3)	1708(1)	22(1)
C(13)	10109(1)	3398(3)	2036(1)	24(1)
C(14)	8779(1)	3474(3)	1904(1)	24(1)

Table 3. Bond lengths [Å] and angles [^{o}] for compound **136**.

O(1)-C(2)	1.3694(15)	O(1)-C(1)	1.4390(18)
O(2)-C(14)	1.3724(16)	O(2)-C(1)	1.4275(16)
O(3)-C(11)	1.2236(15)	C(2)-C(3)	1.3719(19)
C(2)-C(14)	1.3917(19)	C(3)-C(4)	1.3997(17)
C(4)-C(12)	1.3915(18)	C(4)-C(5)	1.5111(18)
C(5)-C(6)	1.5399(18)	C(5)-C(10)	1.5494(17)
C(6)-C(7)	1.5236(19)	C(7)-C(8)	1.523(2)
C(8)-C(9)	1.519(2)	C(9)-C(10)	1.5218(17)
C(10)-C(11)	1.5196(18)	C(11)-C(12)	1.4732(17)
C(12)-C(13)	1.4072(18)	C(13)-C(14)	1.3658(18)
C(2)-O(1)-C(1)	105.89(10)	C(14)-O(2)-C(1)	106.17(10)

O(2)-C(1)-O(1)	107.75(10)	O(1)-C(2)-C(3)	127.05(12)
O(1)-C(2)-C(14)	109.76(11)	C(3)-C(2)-C(14)	123.19(12)
C(2)-C(3)-C(4)	115.34(12)	C(12)-C(4)-C(3)	121.11(12)
C(12)-C(4)-C(5)	111.04(11)	C(3)-C(4)-C(5)	127.65(11)
C(4)-C(5)-C(6)	110.10(11)	C(4)-C(5)-C(10)	102.33(10)
C(6)-C(5)-C(10)	112.24(11)	C(7)-C(6)-C(5)	112.70(11)
C(6)-C(7)-C(8)	109.86(11)	C(9)-C(8)-C(7)	110.57(12)
C(8)-C(9)-C(10)	113.30(11)	C(11)-C(10)-C(9)	116.31(11)
C(11)-C(10)-C(5)	103.89(10)	C(9)-C(10)-C(5)	117.07(10)
O(3)-C(11)-C(12)	127.20(12)	O(3)-C(11)-C(10)	126.09(11)
C(12)-C(11)-C(10)	106.69(10)	C(4)-C(12)-C(13)	123.04(11)
C(4)-C(12)-C(11)	108.97(11)	C(13)-C(12)-C(11)	127.99(11)
C(14)-C(13)-C(12)	114.56(12)	C(13)-C(14)-O(2)	127.60(12)
C(13)-C(14)-C(2)	122.76(12)	O(2)-C(14)-C(2)	109.62(11)

Table 4. Anisotropic displacement parameters ($Å^2$ x 10^3) for compound **136**. The anisotropic displacement factor exponent takes the form: -2 gpi^2 [h^2 a^{*2} U11 + ... + 2 h k a* b* U

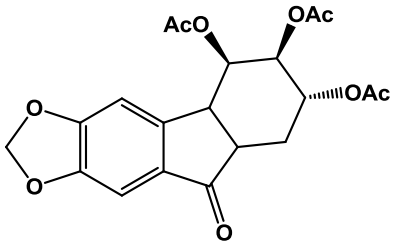
Atom	U11	U22	U33	U23	U13	U12
O(1)	19(1)	47(1)	33(1)	5(1)	-2(1)	-2(1)
O(2)	23(1)	35(1)	34(1)	5(1)	3(1)	-5(1)
O(3)	23(1)	28(1)	29(1)	0(1)	-5(1)	1(1)
C(1)	24(1)	42(1)	40(1)	2(1)	-2(1)	-7(1)
C(2)	20(1)	33(1)	24(1)	-5(1)	-2(1)	0(1)
C(3)	25(1)	31(1)	24(1)	2(1)	-3(1)	3(1)
C(4)	24(1)	24(1)	22(1)	-3(1)	-1(1)	1(1)
C(5)	24(1)	24(1)	26(1)	2(1)	-1(1)	0(1)
C(6)	31(1)	38(1)	24(1)	2(1)	-2(1)	-6(1)
C(7)	37(1)	48(1)	26(1)	2(1)	4(1)	-6(1)
C(8)	29(1)	39(1)	36(1)	2(1)	9(1)	-2(1)
C(9)	23(1)	29(1)	34(1)	4(1)	-2(1)	-4(1)
C(10)	24(1)	23(1)	25(1)	-3(1)	-2(1)	-2(1)
C(11)	23(1)	24(1)	19(1)	-5(1)	-2(1)	0(1)
C(12)	23(1)	23(1)	20(1)	-3(1)	-1(1)	0(1)
C(13)	25(1)	24(1)	21(1)	-1(1)	-1(1)	1(1)
C(14)	23(1)	25(1)	22(1)	-3(1)	3(1)	-3(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$ x 10^3) for compound **136**.

Atom	x	y	z	U(eq)
H(1A)	5916	3077	2139	42
H(1B)	6284	1138	1577	42
H(3)	8519	8256	862	32
H(5)	11079	10501	913	30
H(6A)	11548	5413	344	37
H(6B)	10880	7953	16	37
H(7A)	12964	10122	12	44
H(7B)	13038	7344	-351	44
H(8A)	13990	5306	561	41

H(8B)	14901	7701	334	41
H(9A)	14115	10514	1093	35
H(9B)	14587	7831	1431	35
H(10)	12477	9738	1725	28
H(13)	10500	2183	2326	28

8.3.2. ^{1}H NMR and HRMS of Indanone 141



141

HRMS (+ESI) Found MH^+ 405.1180, $C_{20}H_{21}O_9$ requires *MH* 405.1186 and found MNa^+ 427.0995, $C_{20}H_{20}NaO_9$ requires *MNa* 427.1005.

Confirmation of Expected Formula

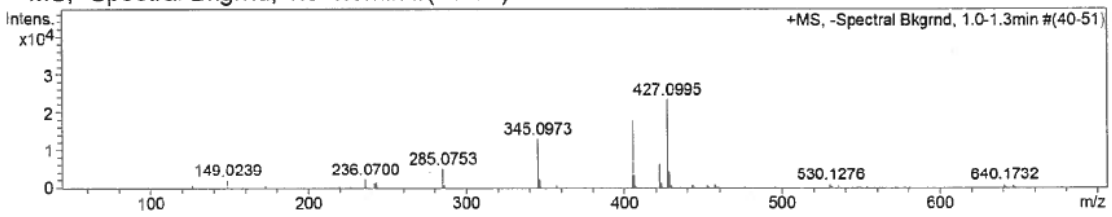
Sample-ID GT02:56:03 — Submitter Gemma Tunbridge

Analysis Name GT02-56-03_199338_98_01_15850.d — Supervisor Lorenzo Caggiano

Method used Confirm Formula Positive 50to500 loop inj.m — Acquisition Date 16/06/2009 14:40:55

Ionisation Mode positive electrospray (ESI)

+MS, -Spectral Bkgrnd, 1.0-1.3min #(40-51)



#	m/z	I	I %	Area	S/N
1	149.0239	2145	9.2	52	82.0
2	236.0700	2299	9.8	34	50.8
3	285.0753	5148	22.0	238	122.6
4	345.0973	13085	55.9	695	575.1
5	346.1013	2349	10.0	124	104.9
6	405.1180	17882	76.4	1039	115.4
7	406.1208	3482	14.9	207	21.8
8	422.1440	6307	26.9	370	30.6
9	427.0995	23419	100.0	1381	126.1
10	428.1038	4402	18.8	275	24.3

lost acetate

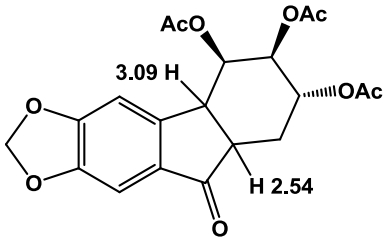
Generate Molecular Formula Parameters

Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
positive	10 ppm	0.05 m/z	0	3	both	true	0.05

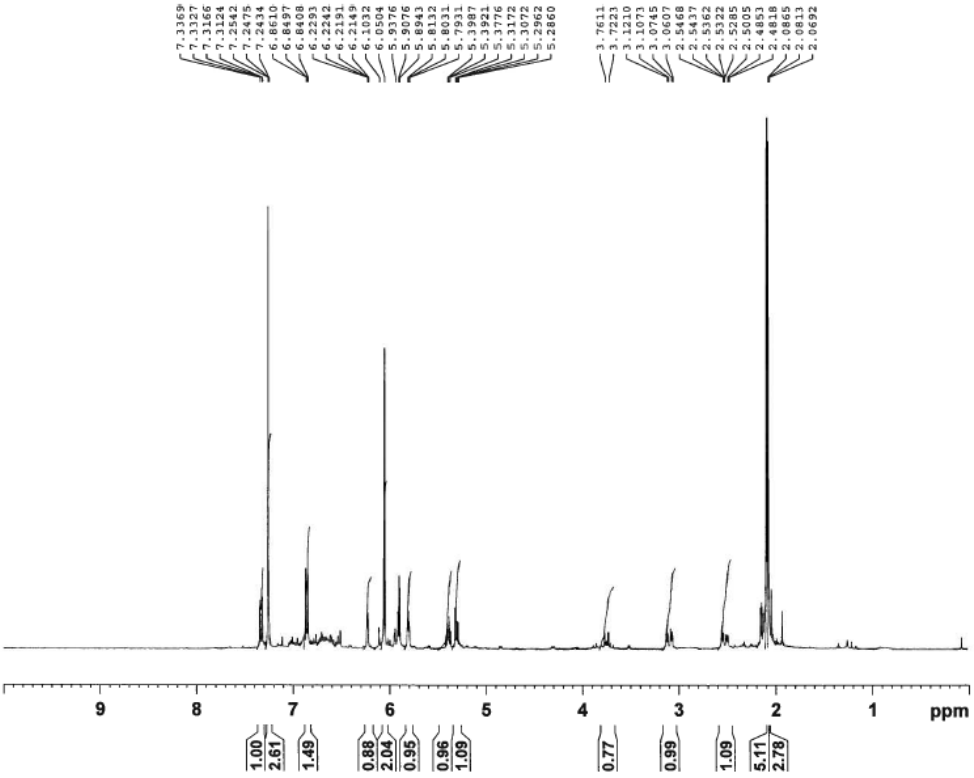
Expected Formula C20 H20 O9 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	405.1180	405.118557	0.10	0.0159	C 20 H 21 O 9
1	427.0995	427.100502	1.10	0.0199	C 20 H 20 Na 1 O 9

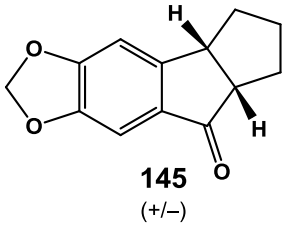
Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication



The presence of two 1H peaks in the ^{1}H NMR at 3.09 and 2.54 ppm again reinforces indanone **141** had been synthesised.

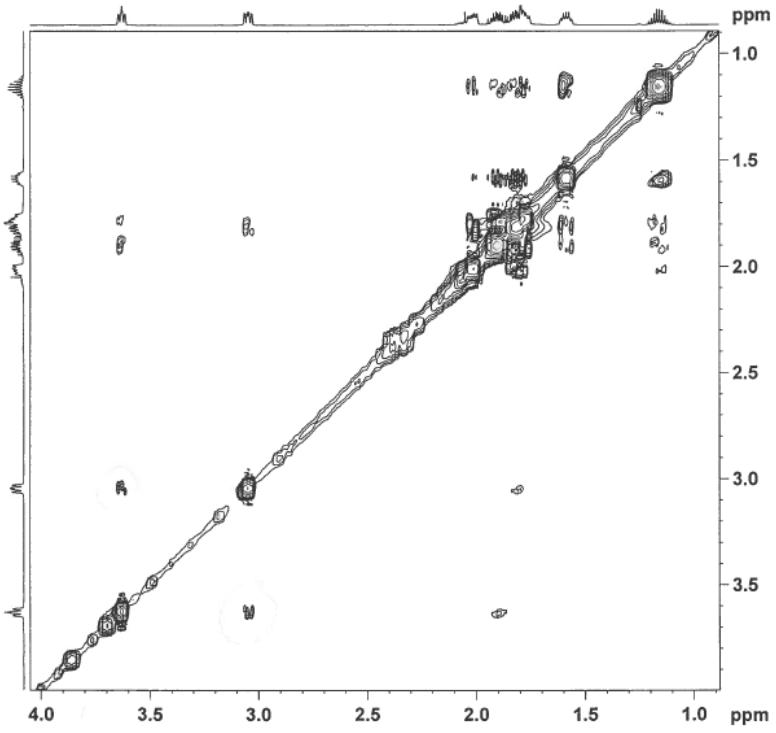


8.3.3. NOESY spectrum of Indanone 145



145
(+/–)

Proton at 3.64-3.60 ppm (1H, m, (CO)C*H*CH) can be seen from proton at 3.04 ppm (1H, ddd, *J* = 10.0, 7.0, 2.0 Hz, (CO)CHC*H*), which reinforces that the indanone **145** has the *cis* configuration.

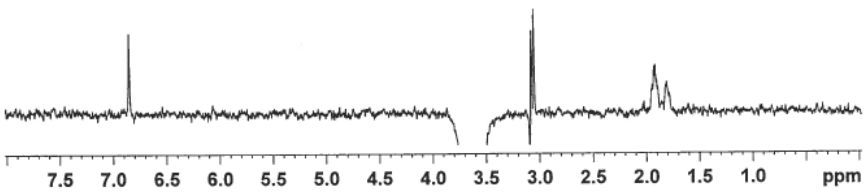


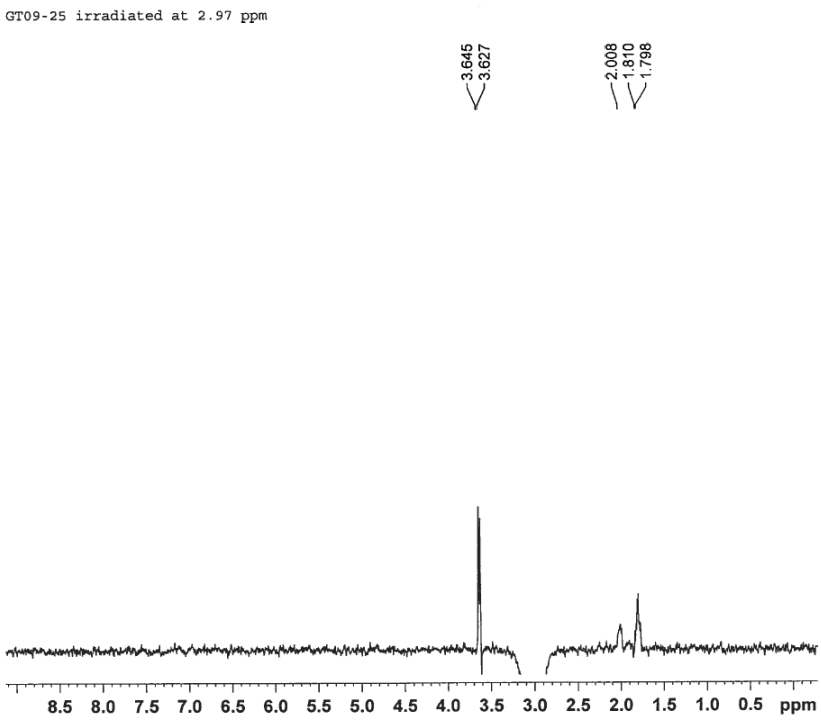
GT09-25 irradiated at 3.56 ppm

6.852

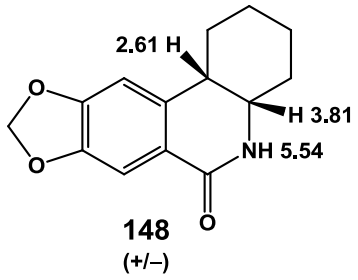
3.077
3.056

2.021
1.929
1.917
1.808



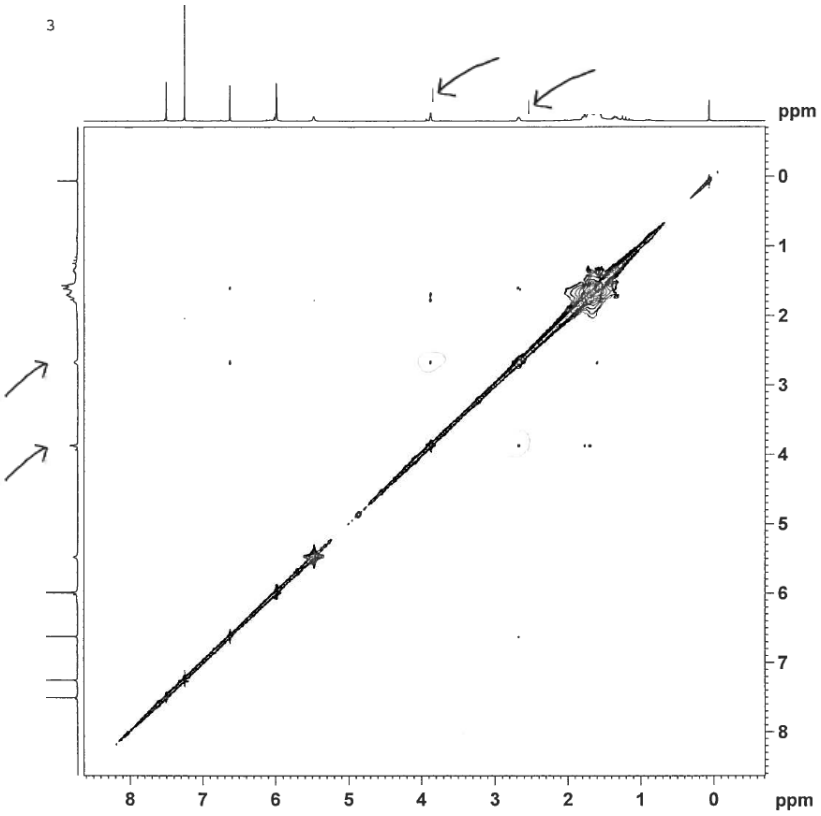


8.3.4. NOESY spectrum of Lactam 148

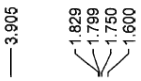


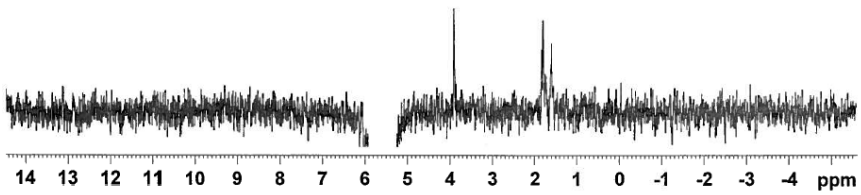
Proton at 3.81 ppm (1H, d, *J* = 4.0 Hz, NC*H*CH) can be seen from proton at 2.61 ppm (1H, br.s. NCHC*H*), which reinforces that the lactam **148** has the *cis* configuration.

Proton at 5.54 (1H, br.s. N*H*) can be seen from proton at 3.81 ppm (1H, d, *J* = 4.0 Hz, NC*H*CH) but not proton at 2.61 ppm (1H, br.s. NCHC*H*), which indicates that the correct regioisomer is shown.

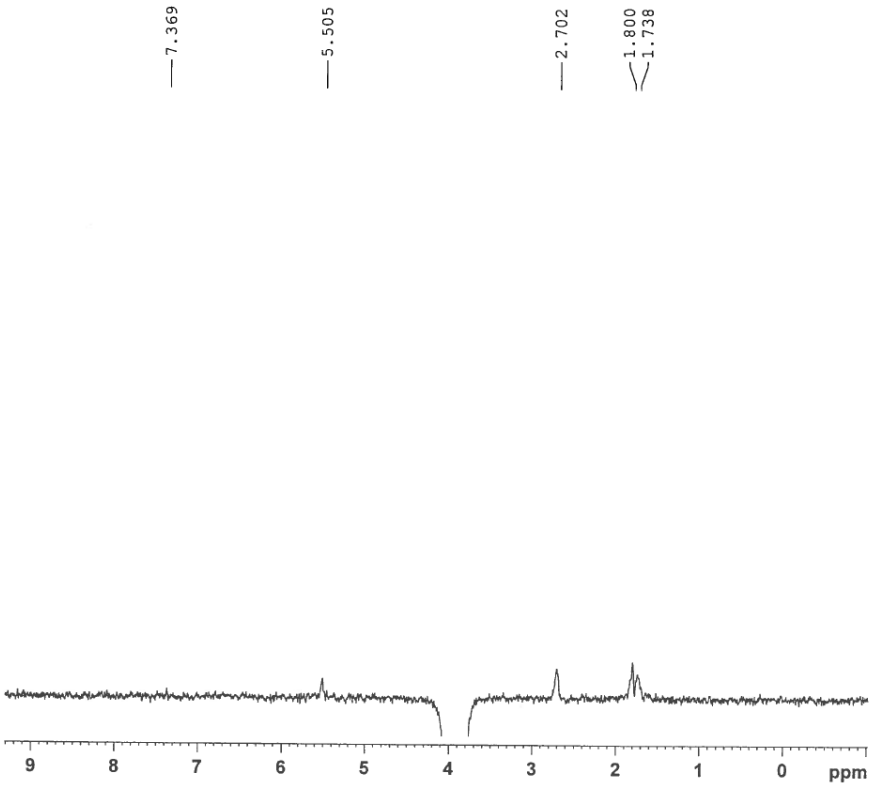


GT09:23:03 irradiated at 5.46 ppm

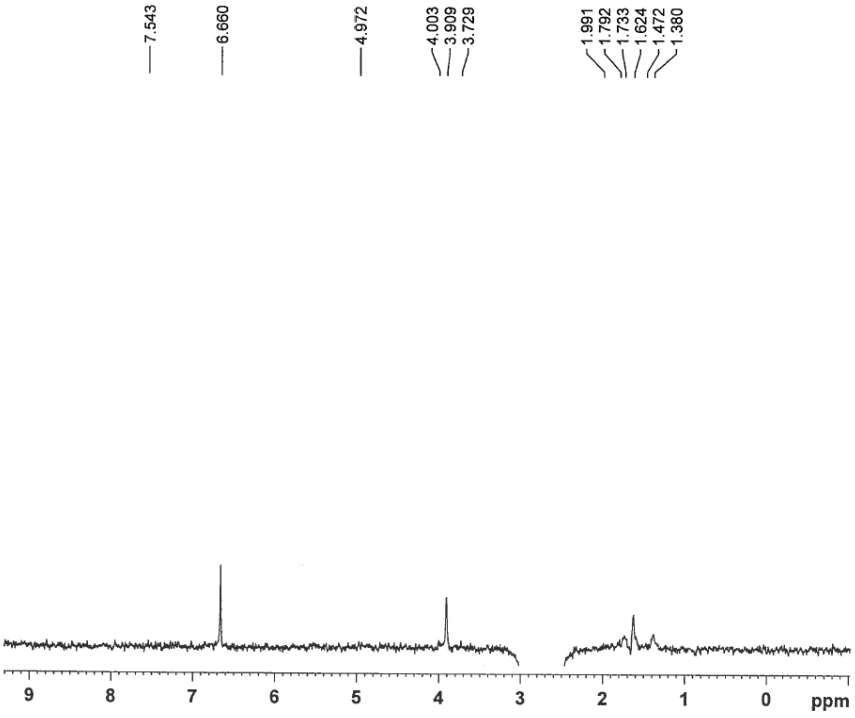




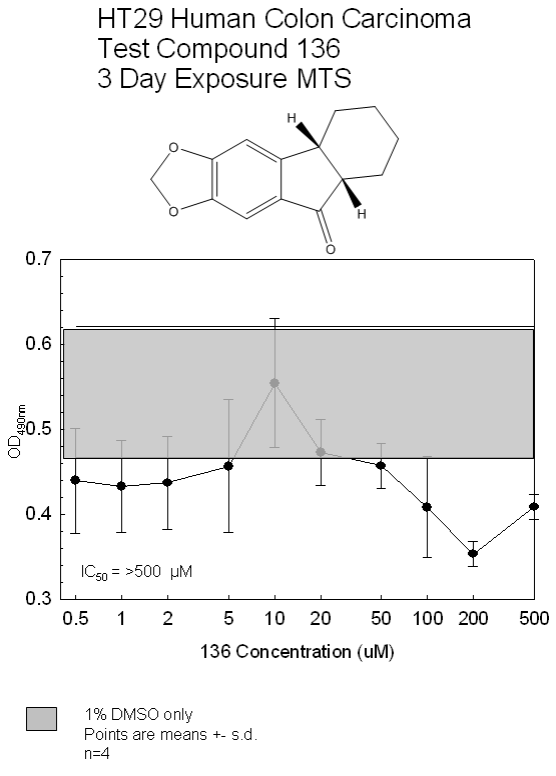
GT09:23:03 irradiated at 3.86 ppm

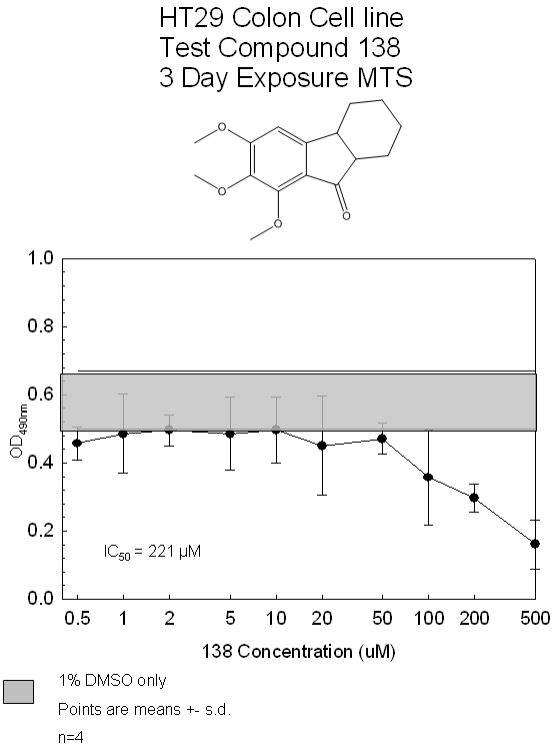


GT09:23:03 irradiated at 2.68 ppm

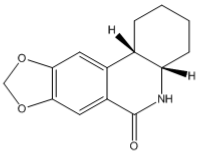


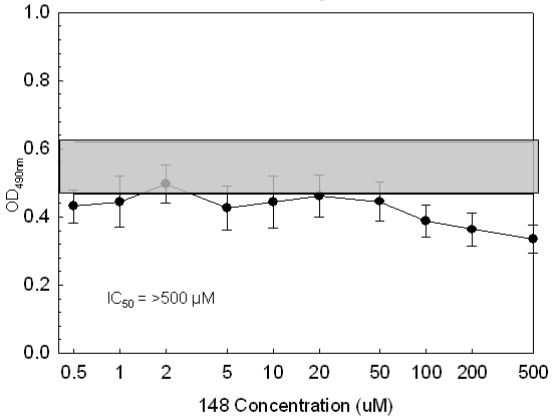
8.3.5. IC_{50} Values of A/B/C Indanones and Lactams in the HT29 Cancer Cell Line





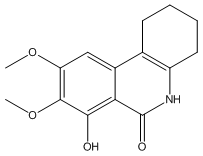
HT29 Colon cell line
Test Compound 148
3 Day Exposure MTS

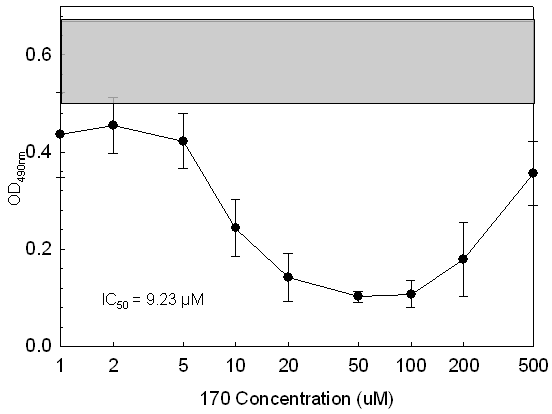




1% DMSO only
Points are means +- s.d.
n=4

HT29 Human Colon Carcinoma
Test Compound 170
3 Day Exposure MTS

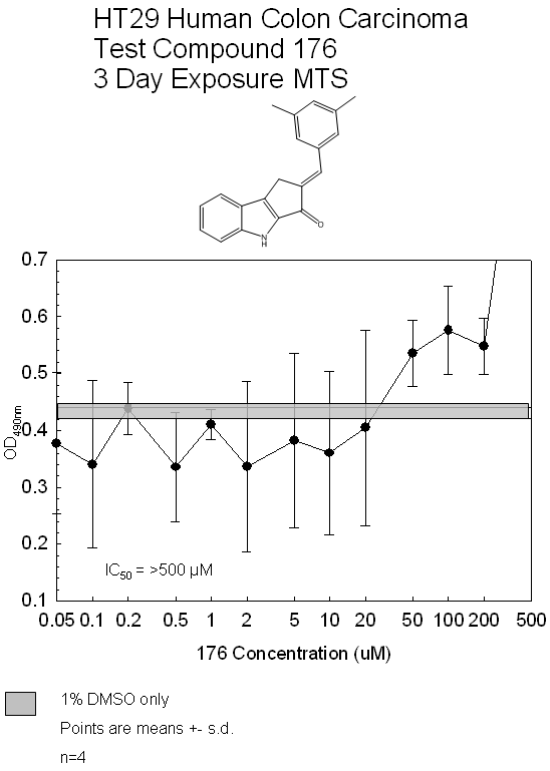


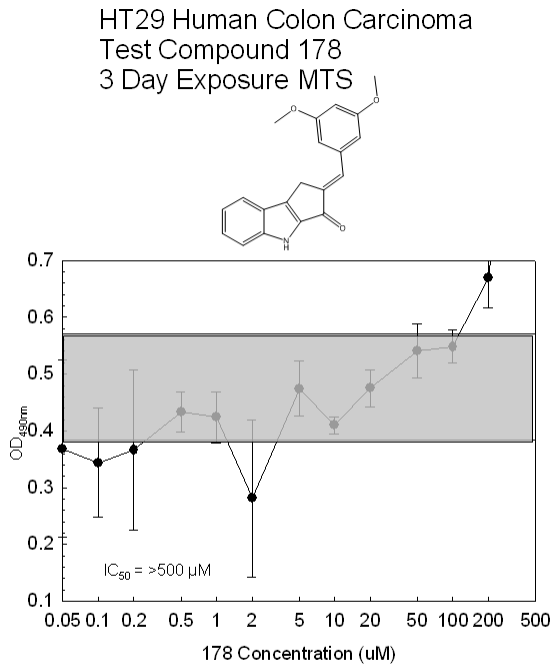


1% DMSO only
Points are means +- s.d.
n=4

8.4. Chapter Five

8.4.1. IC_{50} Values of Indanocine Analogues in HT29 and MDA231 Cancer Cell Lines

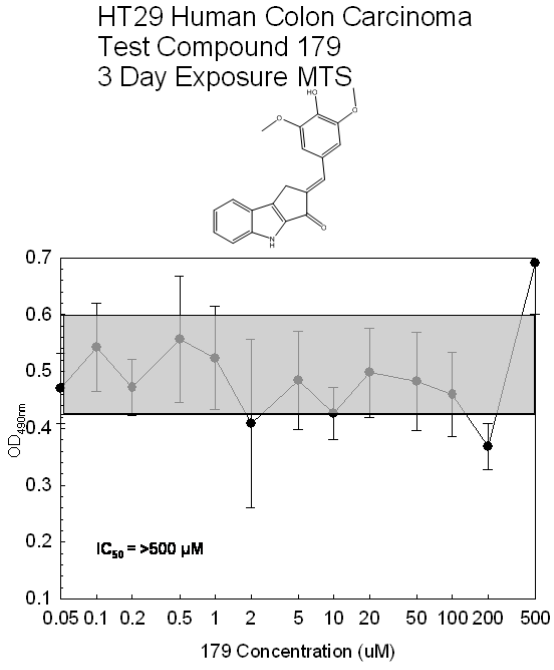




1% DMSO only

Points are means +- s.d.

n=4

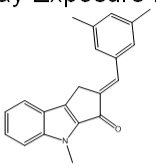


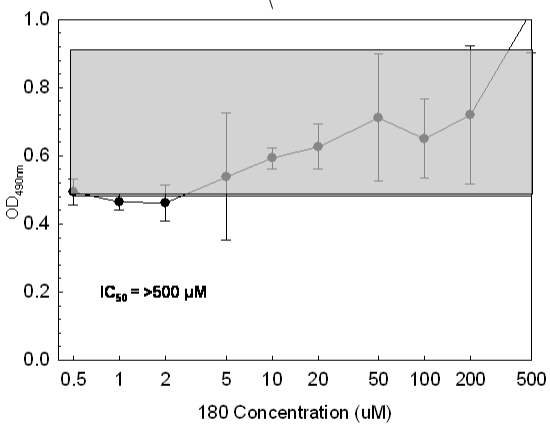
1% DMSO only

Points are means +- s.d.

n=4

HT29 Colon cell line
Test Compound 180
3 Day Exposure MTS



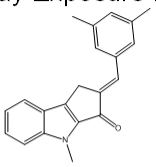


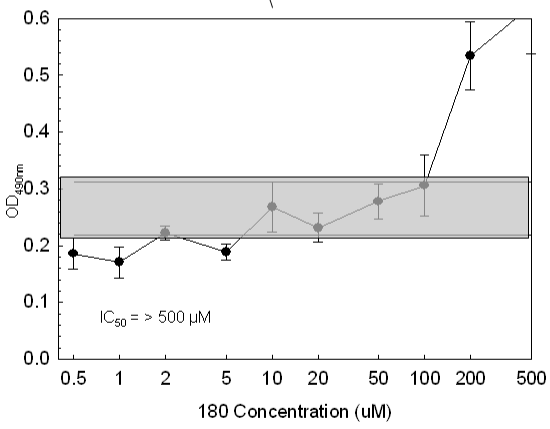
1% DMSO only

Points are means +- s.d.

n=4

MDA Breast cell line
Test Compound 180
3 Day Exposure MTS



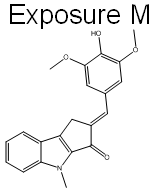


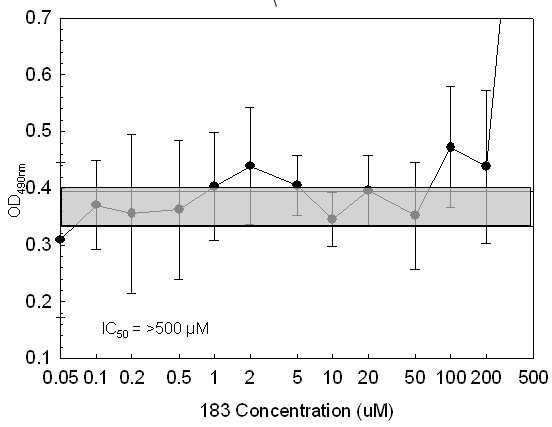
1% DMSO only

Points are means +- s.d.

n=4

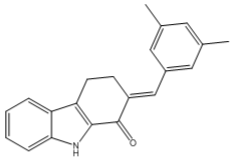
HT29 Human Colon Carcinoma
Test Compound 183
3 Day Exposure MTS

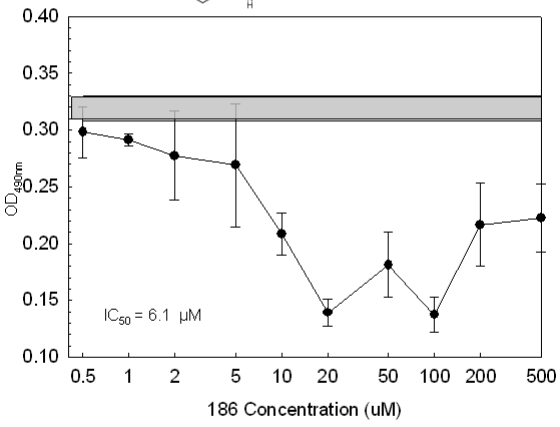




1% DMSO only
Points are means +- s.d.
n=4

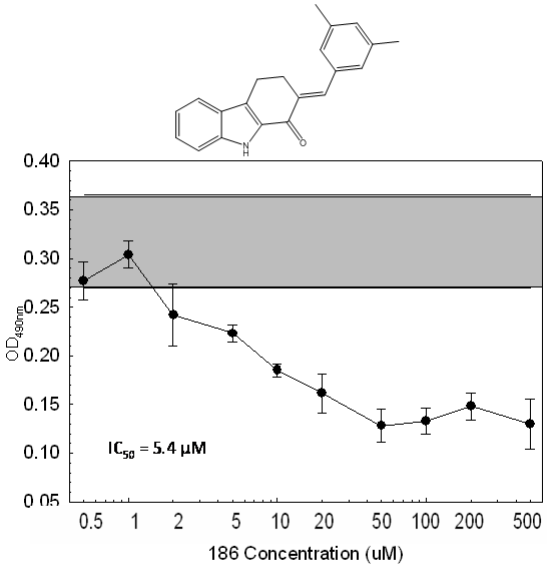
HT29 Human Colon Carcinoma
Test Compound 186
3 Day Exposure MTS





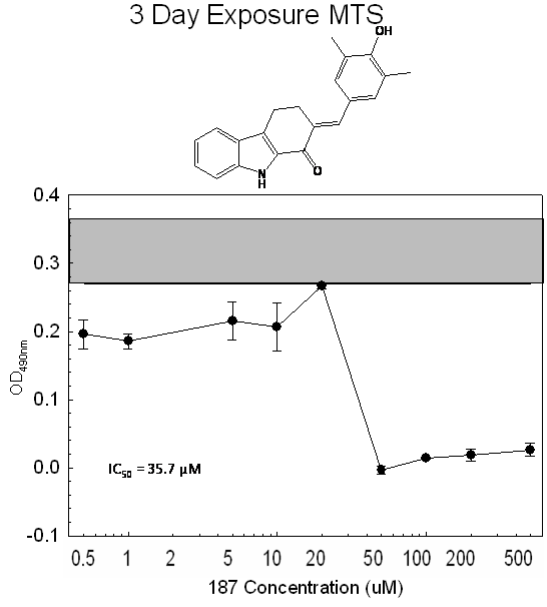
1% DMSO only
Points are means +- s.d.
n=4

MDA231 Breast Carcinoma
Test Compound 186
3 Day Exposure MTS



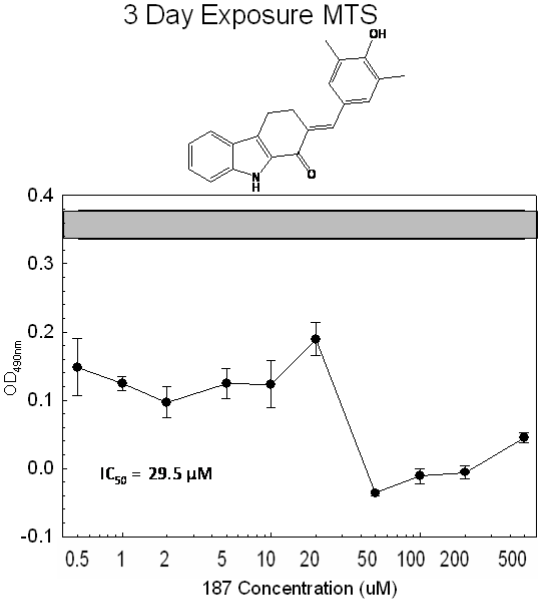
1% DMSO only
Points are means ± s.d
n = 4

MDA231 Breast Carcinoma
Test Compound 187
3 Day Exposure MTS



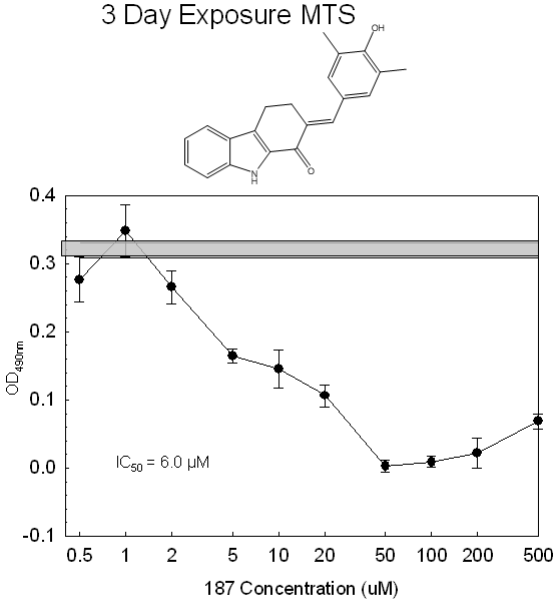
1% DMSO only
Points are means ± s.d
n = 4

MDA231 Breast Carcinoma
Test Compound 187
3 Day Exposure MTS



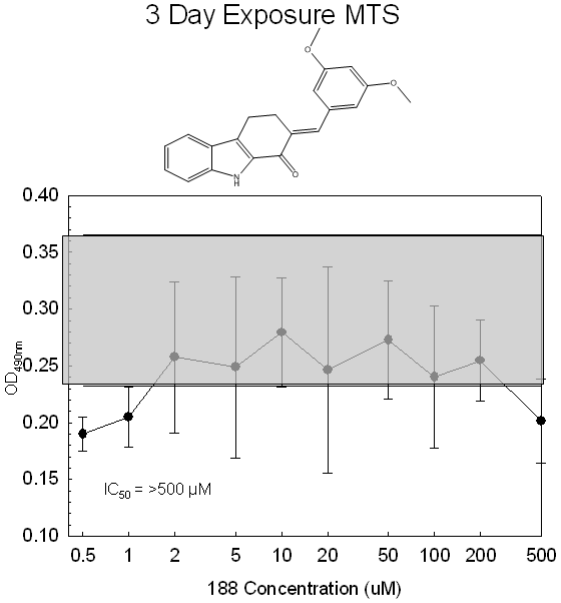
1% DMSO only
Points are means ± s.d
n = 4

HT29 Human Colon Carcinoma
Test Compound 187
3 Day Exposure MTS



1% DMSO only
Points are means +- s.d.
n=4

MDA231 Breast Cell line
Test Compound 188
3 Day Exposure MTS

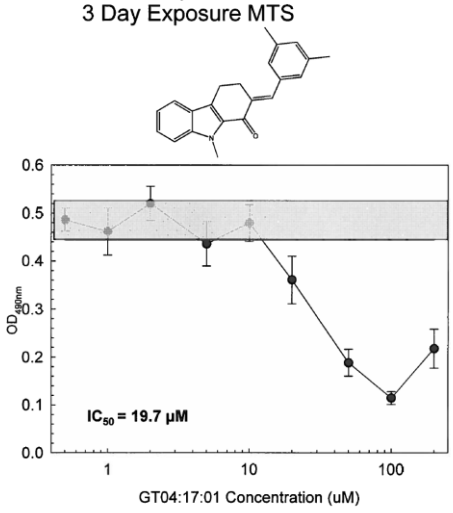


1% DMSO only

Points are means +- s.d.

n=4

HT29 Human Colon Carcinoma
Test Compound 190
3 Day Exposure MTS



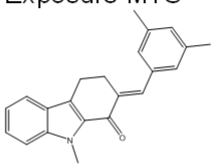
1% DMSO only

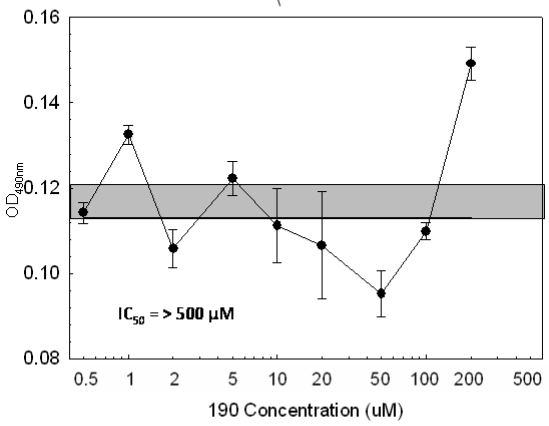
Points are means +- s.d.

n=4

* 500 µM was deleted due to insolubility

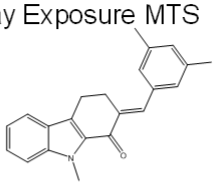
MDA231 Breast Carcinoma
Test Compound 190
3 Day Exposure MTS

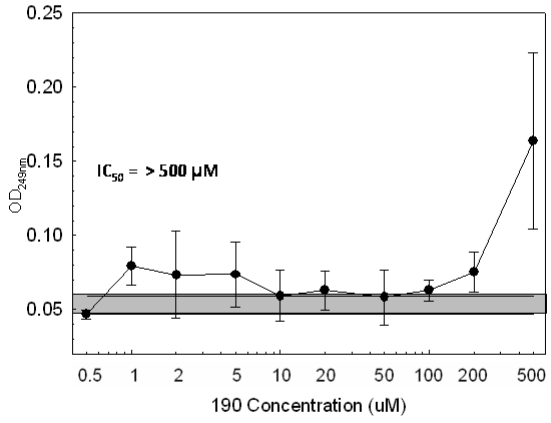




1% DMSO only
Points are means ± s.d
n = 4

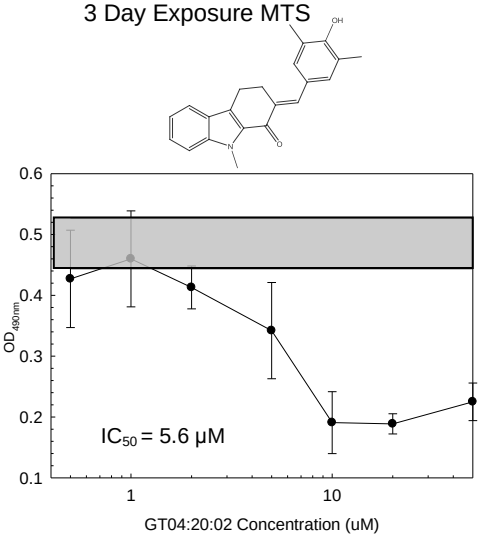
MDA231 Breast Carcinoma
Test Compound 190
3 Day Exposure MTS





1% DMSO only
Points are means ± s.d
n = 4

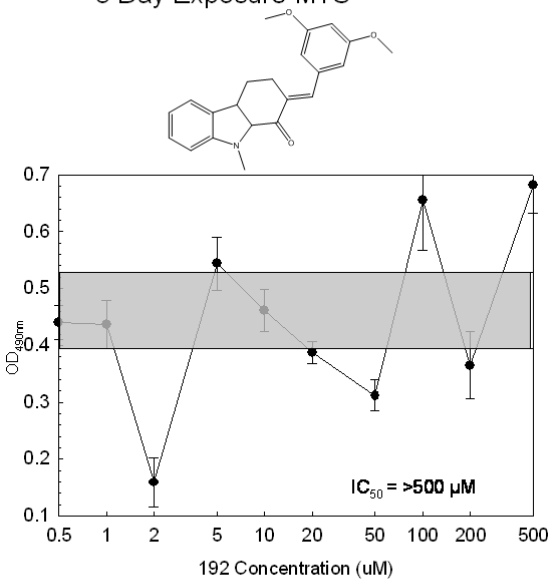
HT29 Human Colon Carcinoma
Test Compound 191
3 Day Exposure MTS



1% DMSO only
Points are means +- s.d.
n=4

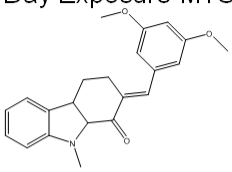
* 500µM, 200µM and 100µM were deleted due to insolubility

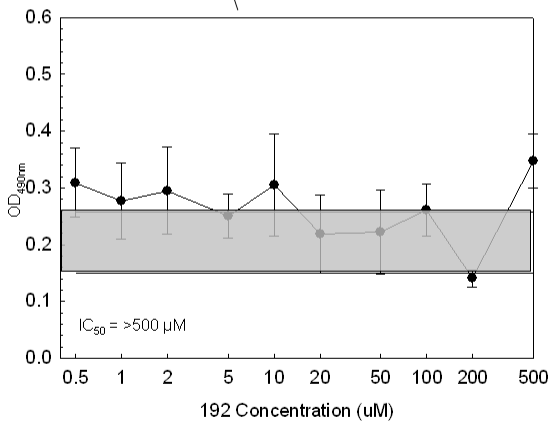
HT29 Human Colon Carcinoma
Test Compound 192
3 Day Exposure MTS



1% DMSO only
Points are means +- s.d.
n=4

MDA231 Breast Cell line
Test Compound 192
3 Day Exposure MTS





1% DMSO only

Points are means +- s.d.

n=4

8.4.2. NCI 60 Cell Line Screen - One-Dose Data

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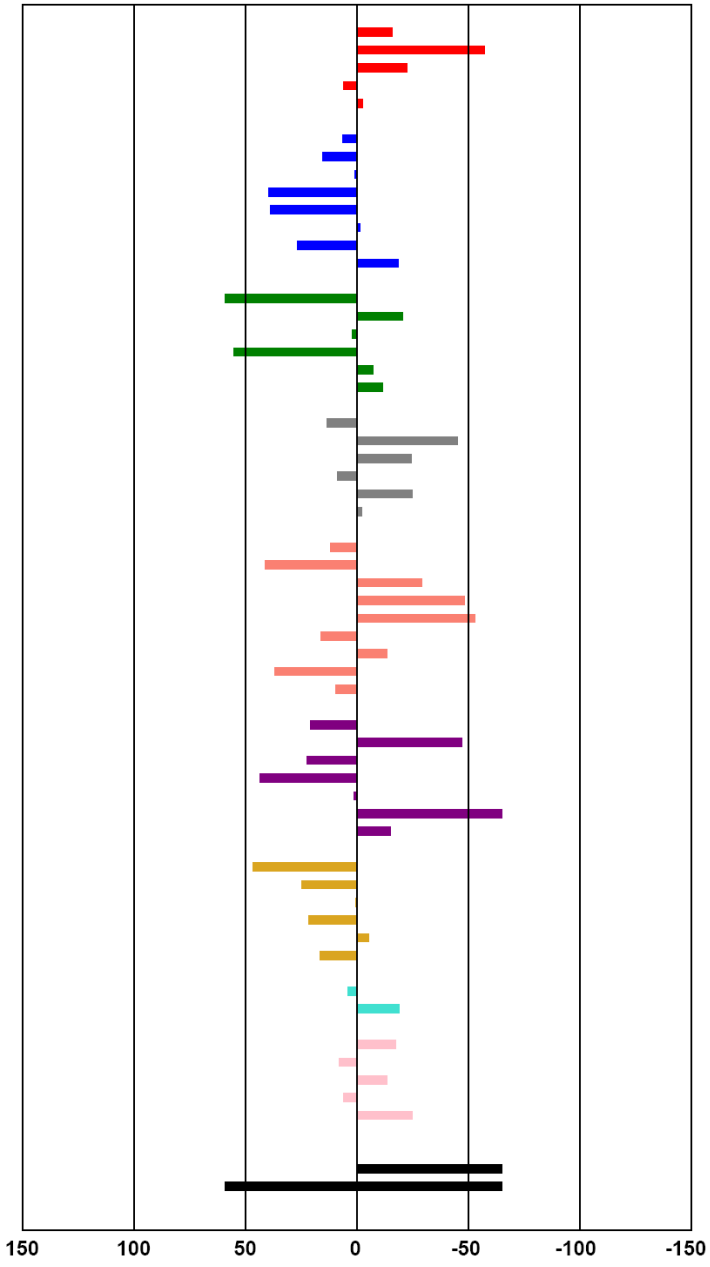
Developmental Therapeutics Program	NSC: D-756592 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 14, 2011
One Dose Mean Graph	Experiment ID: 1103OS19		Report Date: Jul 12, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	17.08
HL-60(TB)	-24.14
K-562	10.60
MOLT-4	38.71
RPMI-8226	30.53
Non-Small Cell Lung Cancer	
A549/ATCC	39.12
EKVX	47.96
HOP-62	33.49
HOP-92	72.36
NCI-H226	71.60
NCI-H23	31.67
NCI-H322M	59.47
NCI-H460	14.59
Colon Cancer	
COLO 205	91.69
HCT-116	12.39
HCT-15	34.73
HT29	87.90
KM12	25.86
SW-620	21.44
CNS Cancer	
SF-268	46.23
SF-295	-11.95
SF-539	8.62
SNB-19	41.49
SNB-75	8.29
U251	30.73
Melanoma	
LOX IMVI	44.70
MALME-3M	74.01
M14	3.81
MDA-MB-435	-15.16
SK-MEL-2	-19.77
SK-MEL-28	49.00
SK-MEL-5	19.67
UACC-257	69.60
UACC-62	42.41
Ovarian Cancer	
IGROV1	53.41
OVCAR-3	-13.93
OVCAR-4	55.18
OVCAR-5	76.33
OVCAR-8	34.07
NCI/ADR-RES	-32.10
SK-OV-3	17.90
Renal Cancer	
A498	79.25
ACHN	57.34
CAKI-1	33.28
SN12C	54.24
TK-10	27.64
UO-31	49.38
Prostate Cancer	
PC-3	36.69
DU-145	13.93
Breast Cancer	
MCF7	15.58
MDA-MB-231/ATCC	40.56
HS 578T	19.66
T-47D	38.60
MDA-MB-468	8.06
Mean	32.85
Delta	64.95
Range	123.79

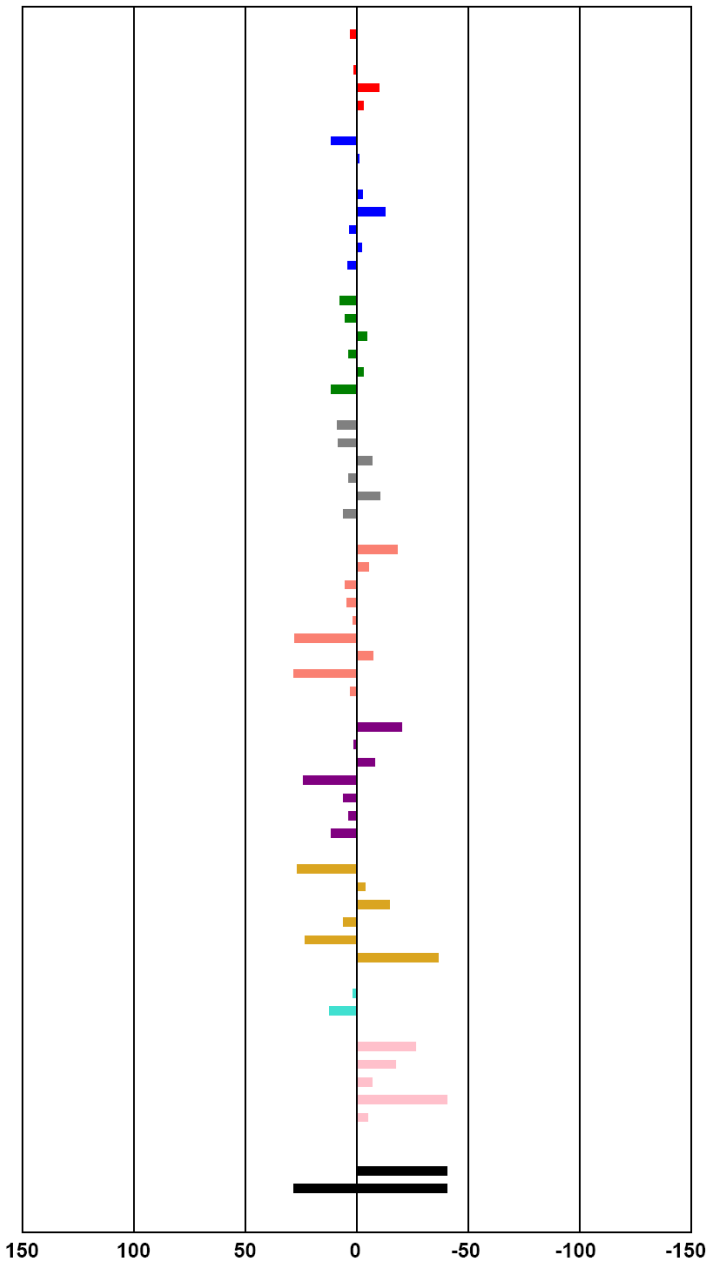
Mean Growth Percent - Growth Percent



Developmental Therapeutics Program	NSC: D-756591 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 14, 2011
One Dose Mean Graph	Experiment ID: 1103OS19		Report Date: Jul 12, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	97.87
HL-60(TB)	94.97
K-562	96.04
MOLT-4	85.45
RPMI-8226	92.26
Non-Small Cell Lung Cancer	
A549/ATCC	106.38
EKVX	94.35
HOP-62	95.11
HOP-92	92.80
NCI-H226	82.57
NCI-H23	98.31
NCI-H322M	92.95
NCI-H460	99.01
Colon Cancer	
COLO 205	102.42
HCT-116	100.05
HCT-15	90.73
HT29	98.42
KM12	92.40
SW-620	106.55
CNS Cancer	
SF-268	103.64
SF-295	103.41
SF-539	88.27
SNB-19	98.40
SNB-75	84.82
U251	101.00
Melanoma	
LOX IMVI	77.02
MALME-3M	90.01
M14	100.30
MDA-MB-435	99.34
SK-MEL-2	96.70
SK-MEL-28	122.90
SK-MEL-5	88.15
UACC-257	123.25
UACC-62	97.83
Ovarian Cancer	
IGROV1	75.02
OVCAR-3	96.10
OVCAR-4	87.40
OVCAR-5	118.91
OVCAR-8	100.99
NCI/ADR-RES	98.73
SK-OV-3	106.19
Renal Cancer	
A498	121.56
ACHN	91.57
CAKI-1	80.61
SN12C	100.90
TK-10	118.06
UO-31	58.63
Prostate Cancer	
PC-3	96.65
DU-145	107.25
Breast Cancer	
MCF7	68.82
MDA-MB-231/ATCC	77.67
HS 578T	88.21
T-47D	54.84
MDA-MB-468	90.30
Mean	95.04
Delta	40.20
Range	68.41

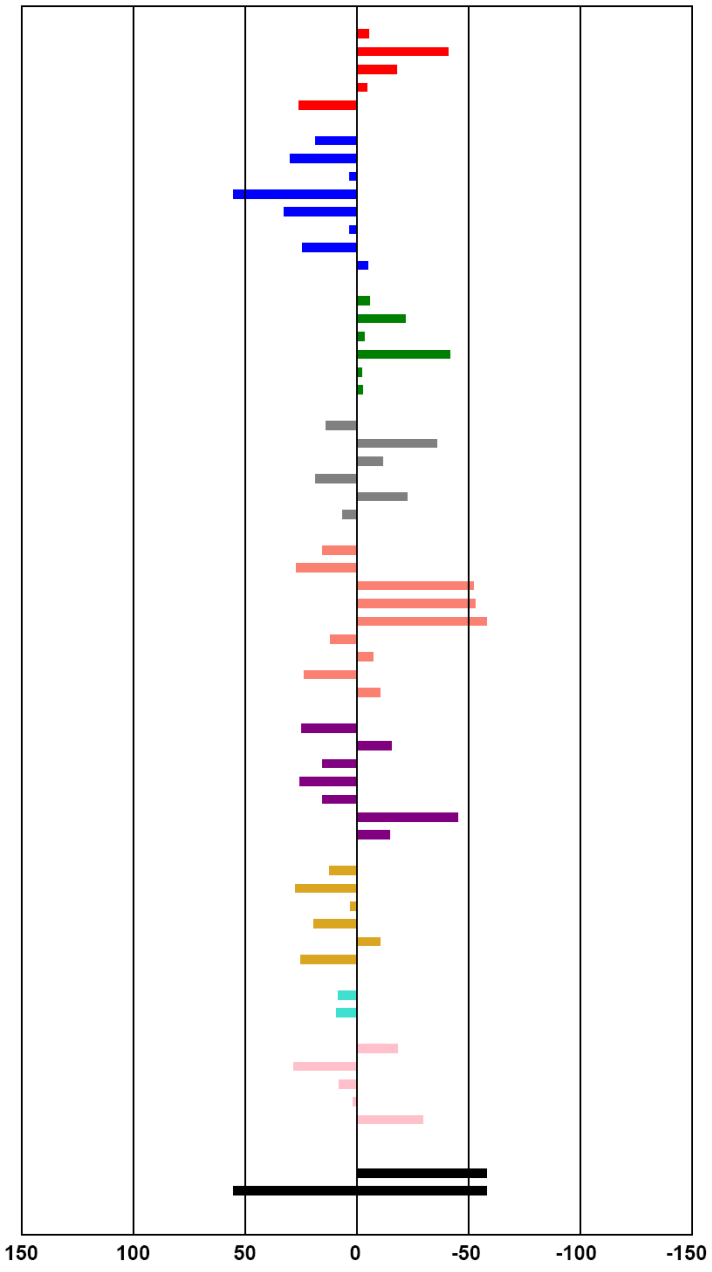
Mean Growth Percent - Growth Percent



Developmental Therapeutics Program	NSC: D-756584 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 14, 2011
One Dose Mean Graph	Experiment ID: 1103OS19		Report Date: Jul 12, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	21.76
HL-60(TB)	-13.97
K-562	9.10
MOLT-4	22.49
RPMI-8226	52.61
Non-Small Cell Lung Cancer	
A549/ATCC	45.05
EKVX	56.57
HOP-62	29.96
HOP-92	81.96
NCI-H226	59.20
NCI-H23	30.04
NCI-H322M	50.86
NCI-H460	22.11
Colon Cancer	
COLO 205	21.47
HCT-116	5.11
HCT-15	23.49
HT29	-14.65
KM12	24.79
SW-620	24.31
CNS Cancer	
SF-268	40.58
SF-295	-8.71
SF-539	15.35
SNB-19	45.09
SNB-75	4.60
U251	32.95
Melanoma	
LOX IMVI	42.07
MALME-3M	53.56
M14	-25.18
MDA-MB-435	-26.10
SK-MEL-2	-30.98
SK-MEL-28	38.46
SK-MEL-5	19.91
UACC-257	50.33
UACC-62	16.66
Ovarian Cancer	
IGROV1	51.25
OVCAR-3	11.49
OVCAR-4	42.00
OVCAR-5	52.09
OVCAR-8	41.87
NCI/ADR-RES	-18.18
SK-OV-3	12.25
Renal Cancer	
A498	38.85
ACHN	54.01
CAKI-1	29.40
SN12C	45.76
TK-10	16.62
UO-31	51.82
Prostate Cancer	
PC-3	34.78
DU-145	35.60
Breast Cancer	
MCF7	8.95
MDA-MB-231/ATCC	55.03
HS 578T	34.56
T-47D	28.20
MDA-MB-468	-2.38
Mean	26.76
Delta	57.74
Range	112.94

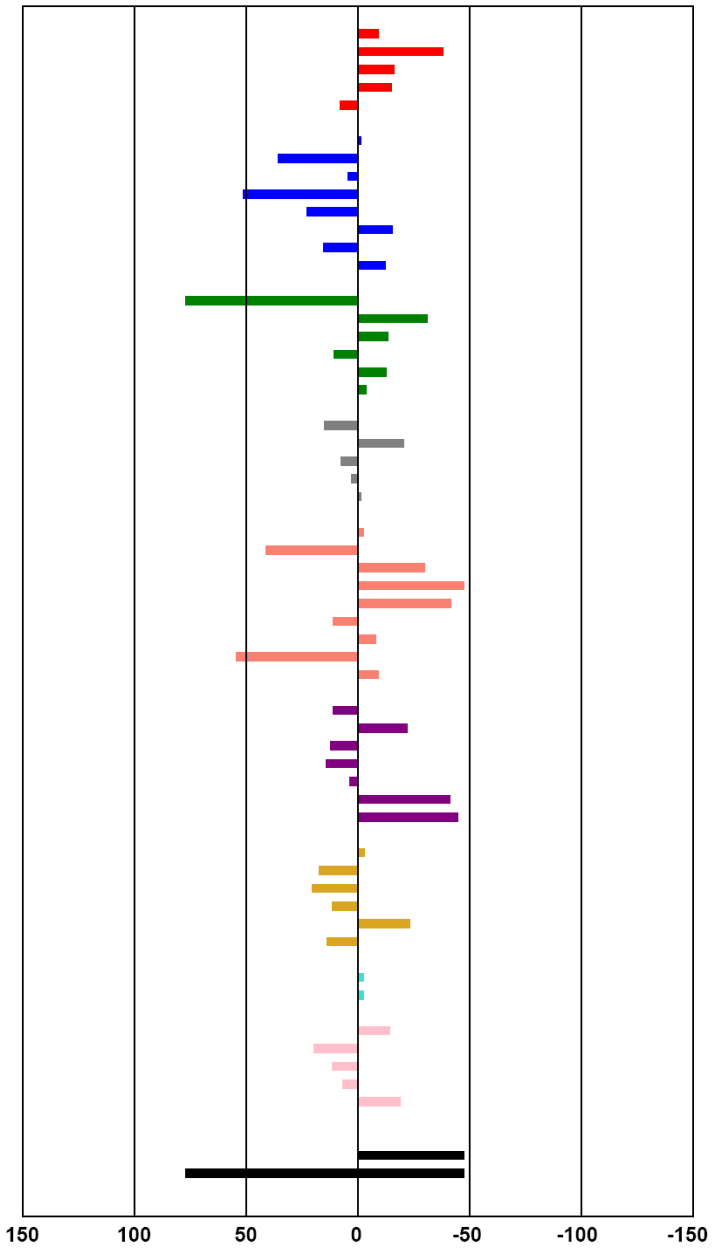
Mean Growth Percent - Growth Percent



Developmental Therapeutics Program	NSC: D-756586 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 14, 2011
One Dose Mean Graph	Experiment ID: 1103OS19		Report Date: Jul 12, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	13.27
HL-60(TB)	-15.76
K-562	6.02
MOLT-4	7.47
RPMI-8226	29.96
Non-Small Cell Lung Cancer	
A549/ATCC	20.82
EKVX	57.84
HOP-62	26.63
HOP-92	73.21
NCI-H226	44.62
NCI-H23	7.11
NCI-H322M	37.38
NCI-H460	10.02
Colon Cancer	
COLO 205	99.09
HCT-116	-8.58
HCT-15	8.91
HT29	32.79
KM12	9.69
SW-620	18.62
CNS Cancer	
SF-268	36.97
SF-539	1.87
SNB-19	29.38
SNB-75	24.81
U251	21.16
Melanoma	
LOX IMVI	19.71
MALME-3M	63.29
M14	-7.72
MDA-MB-435	-25.27
SK-MEL-2	-19.41
SK-MEL-28	32.89
SK-MEL-5	14.15
UACC-257	76.54
UACC-62	13.18
Ovarian Cancer	
IGROV1	33.00
OVCAR-3	0.27
OVCAR-4	34.33
OVCAR-5	36.36
OVCAR-8	25.54
NCI/ADR-RES	-18.79
SK-OV-3	-22.30
Renal Cancer	
A498	19.55
ACHN	39.41
CAKI-1	42.45
SN12C	33.52
TK-10	-0.92
UO-31	36.00
Prostate Cancer	
PC-3	19.79
DU-145	19.65
Breast Cancer	
MCF7	8.15
MDA-MB-231/ATCC	41.57
HS 578T	33.42
T-47D	28.71
MDA-MB-468	3.24
Mean	22.14
Delta	47.41
Range	124.36

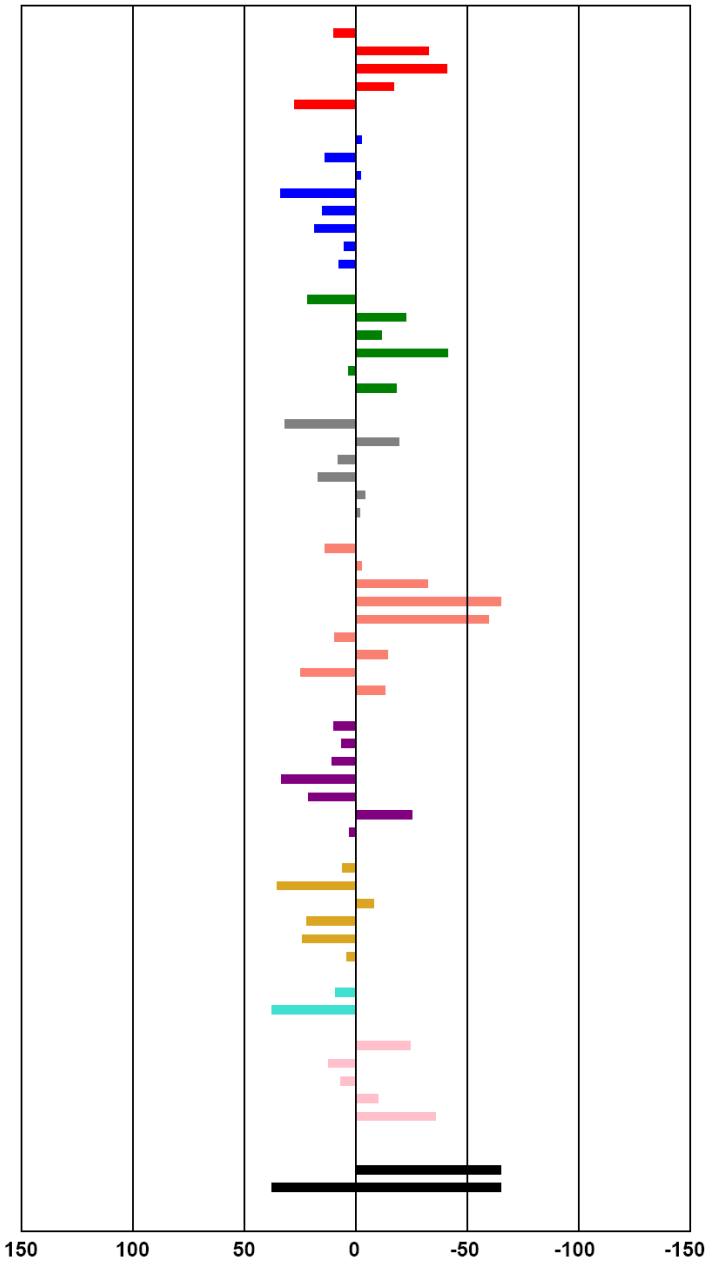
Mean Growth Percent - Growth Percent



Developmental Therapeutics Program	NSC: D-756585 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 14, 2011
One Dose Mean Graph	Experiment ID: 1103OS19		Report Date: Jul 12, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	66.66
HL-60(TB)	24.37
K-562	16.24
MOLT-4	40.35
RPMI-8226	84.32
Non-Small Cell Lung Cancer	
A549/ATCC	54.44
EKVX	70.53
HOP-62	55.14
HOP-92	90.65
NCI-H226	71.79
NCI-H23	75.45
NCI-H322M	62.15
NCI-H460	64.28
Colon Cancer	
COLO 205	78.52
HCT-116	34.62
HCT-15	45.83
HT29	16.08
KM12	60.06
SW-620	39.06
CNS Cancer	
SF-268	88.65
SF-295	37.84
SF-539	64.89
SNB-19	73.80
SNB-75	53.20
U251	55.42
Melanoma	
LOX IMVI	70.58
MALME-3M	54.74
M14	25.03
MDA-MB-435	-7.74
SK-MEL-2	-2.46
SK-MEL-28	66.17
SK-MEL-5	42.85
UACC-257	81.62
UACC-62	44.22
Ovarian Cancer	
IGROV1	66.87
OVCAR-3	63.23
OVCAR-4	67.52
OVCAR-5	90.23
OVCAR-8	78.24
NCI/ADR-RES	31.86
SK-OV-3	59.58
Renal Cancer	
A498	62.86
ACHN	92.13
CAKI-1	49.16
SN12C	78.85
TK-10	80.75
UO-31	60.91
Prostate Cancer	
PC-3	66.00
DU-145	94.34
Breast Cancer	
MCF7	32.63
MDA-MB-231/ATCC	69.12
HS 578T	63.75
T-47D	47.32
MDA-MB-468	21.60
Mean	56.97
Delta	64.71
Range	102.08

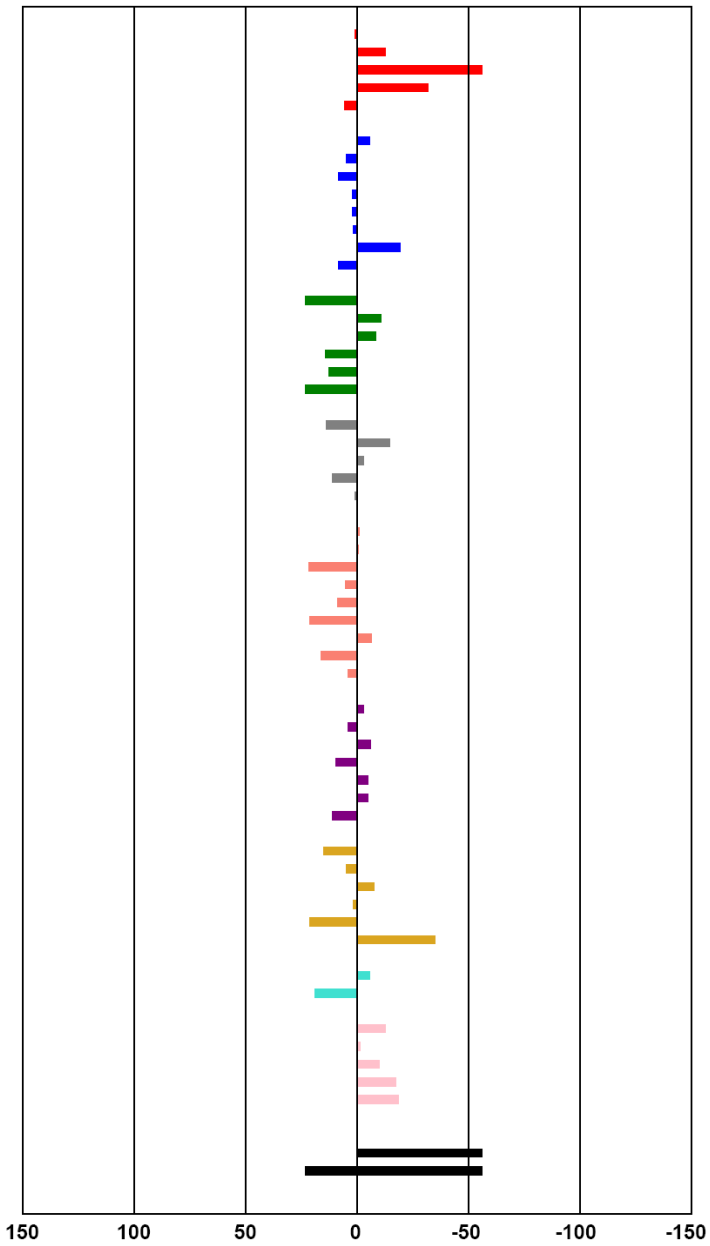
Mean Growth Percent - Growth Percent



Developmental Therapeutics Program	NSC: D-756587 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 14, 2011
One Dose Mean Graph	Experiment ID: 1103OS19		Report Date: Jul 12, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	88.78
HL-60(TB)	75.62
K-562	32.06
MOLT-4	56.51
RPMI-8226	93.71
Non-Small Cell Lung Cancer	
A549/ATCC	82.79
EKVX	92.66
HOP-62	96.16
HOP-92	89.95
NCI-H226	89.99
NCI-H23	89.74
NCI-H322M	68.80
NCI-H460	96.33
Colon Cancer	
COLO 205	111.21
HCT-116	77.46
HCT-15	80.04
HT29	102.21
KM12	100.69
SW-620	111.27
CNS Cancer	
SF-268	101.67
SF-295	73.57
SF-539	85.27
SNB-19	99.13
U251	88.76
Melanoma	
LOX IMVI	87.17
MALME-3M	87.60
M14	109.72
MDA-MB-435	93.27
SK-MEL-2	96.69
SK-MEL-28	109.19
SK-MEL-5	81.73
UACC-257	103.89
UACC-62	91.91
Ovarian Cancer	
IGROV1	85.24
OVCAR-3	92.07
OVCAR-4	82.35
OVCAR-5	97.59
OVCAR-8	83.45
NCI/ADR-RES	83.35
SK-OV-3	98.88
Renal Cancer	
A498	102.94
ACHN	92.76
CAKI-1	80.77
SN12C	89.44
TK-10	109.33
UO-31	53.23
Prostate Cancer	
PC-3	82.42
DU-145	106.92
Breast Cancer	
MCF7	75.42
MDA-MB-231/ATCC	87.07
HS 578T	78.38
T-47D	70.73
MDA-MB-468	69.54
Mean	88.06
Delta	56.00
Range	79.21

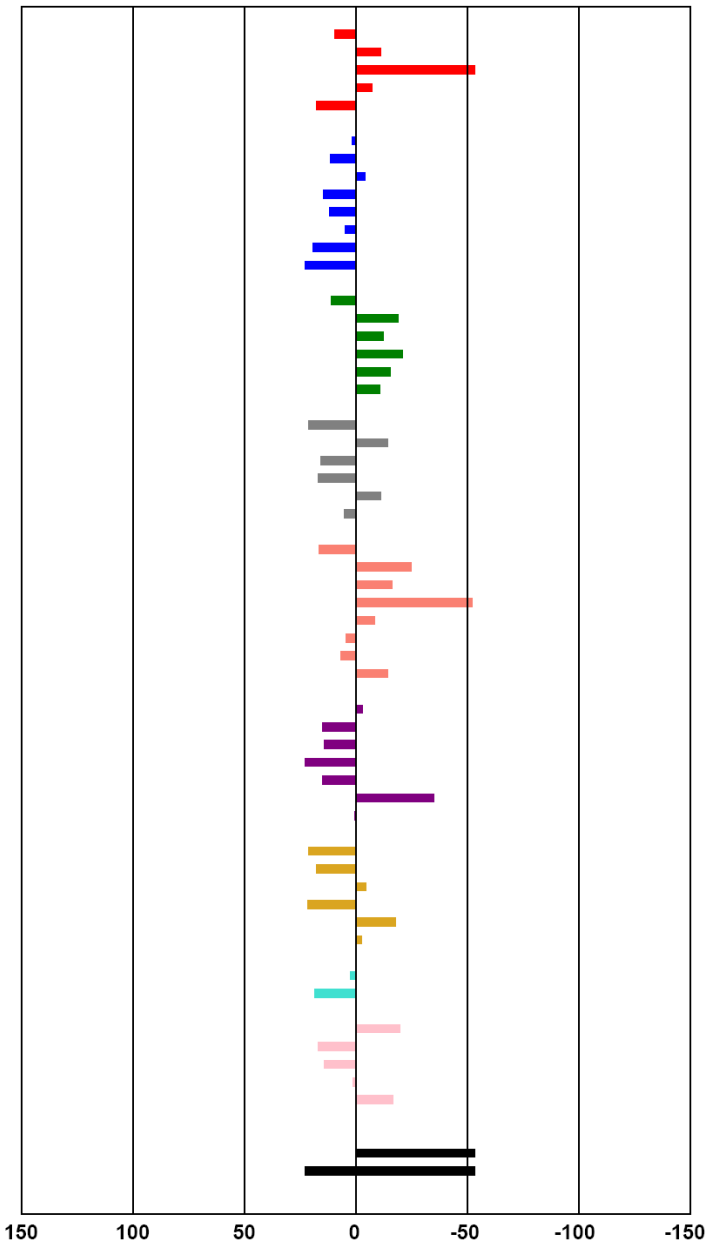
Mean Growth Percent - Growth Percent



Developmental Therapeutics Program One Dose Mean Graph	NSC: D-756588 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 14, 2011
	Experiment ID: 1103OS19		Report Date: Jul 12, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	88.49
HL-60(TB)	68.32
K-562	26.01
MOLT-4	72.27
RPMI-8226	96.84
Non-Small Cell Lung Cancer	
A549/ATCC	80.80
EKVX	90.48
HOP-62	75.23
HOP-92	93.67
NCI-H226	90.87
NCI-H23	84.09
NCI-H322M	98.17
NCI-H460	101.68
Colon Cancer	
COLO 205	90.21
HCT-116	60.38
HCT-15	67.00
HT29	58.46
KM12	64.12
SW-620	68.82
CNS Cancer	
SF-268	100.47
SF-295	65.26
SF-539	94.85
SNB-19	95.90
SNB-75	68.47
U251	84.41
Melanoma	
LOX IMVI	95.65
MALME-3M	54.46
M14	63.33
MDA-MB-435	27.48
SK-MEL-28	71.20
SK-MEL-5	83.65
UACC-257	85.72
UACC-62	65.08
Ovarian Cancer	
IGROV1	76.63
OVCAR-3	93.95
OVCAR-4	93.34
OVCAR-5	101.75
OVCAR-8	93.88
NCI/ADR-RES	44.66
SK-OV-3	79.48
Renal Cancer	
A498	100.33
ACHN	96.78
CAKI-1	74.95
SN12C	100.52
TK-10	61.50
UO-31	77.02
Prostate Cancer	
PC-3	81.73
DU-145	97.71
Breast Cancer	
MCF7	59.84
MDA-MB-231/ATCC	95.91
HS 578T	93.27
T-47D	80.57
MDA-MB-468	62.93
Mean	79.22
Delta	53.21
Range	75.74

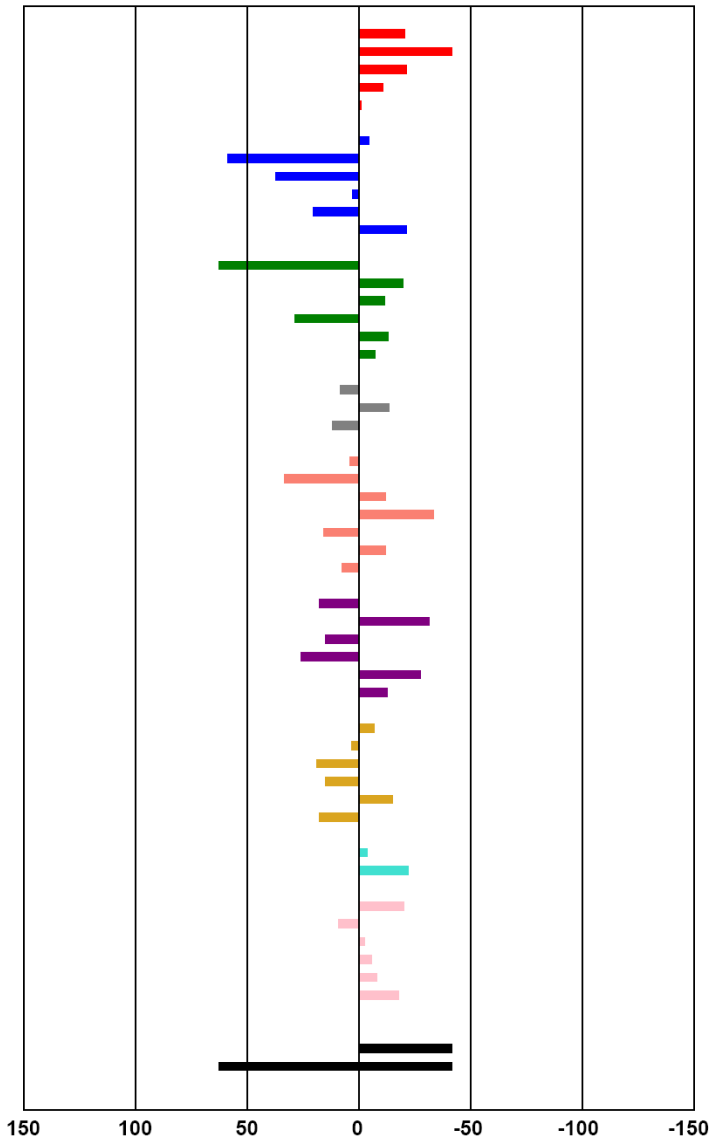
Mean Growth Percent - Growth Percent



Developmental Therapeutics Program One Dose Mean Graph	NSC: D-756590 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 14, 2011
	Experiment ID: 1103OS19		Report Date: Jul 12, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	14.02
HL-60(TB)	-7.09
K-562	13.22
MOLT-4	23.53
RPMI-8226	33.46
Non-Small Cell Lung Cancer	
HOP-62	29.85
HOP-92	92.70
NCI-H226	71.48
NCI-H23	37.01
NCI-H322M	54.37
NCI-H460	13.29
Colon Cancer	
COLO 205	96.51
HCT-116	14.61
HCT-15	22.73
HT29	62.60
KM12	21.33
SW-620	27.19
CNS Cancer	
SF-268	42.47
SF-539	21.06
SNB-19	45.94
Melanoma	
LOX IMVI	38.09
MALME-3M	67.41
M14	22.66
SK-MEL-2	1.08
SK-MEL-28	49.93
SK-MEL-5	22.66
UACC-62	41.69
Ovarian Cancer	
IGROV1	51.72
OVCAR-3	2.80
OVCAR-4	49.04
OVCAR-5	59.93
NCI/ADR-RES	6.70
SK-OV-3	21.75
Renal Cancer	
786-0	27.61
A498	37.18
ACHN	53.07
SN12C	48.89
TK-10	19.49
UO-31	51.84
Prostate Cancer	
PC-3	30.54
DU-145	12.28
Breast Cancer	
MCF7	14.21
MDA-MB-231/ATCC	43.24
HS 578T	31.76
BT-549	28.68
T-47D	26.53
MDA-MB-468	16.51
Mean	34.20
Delta	41.29
Range	103.60

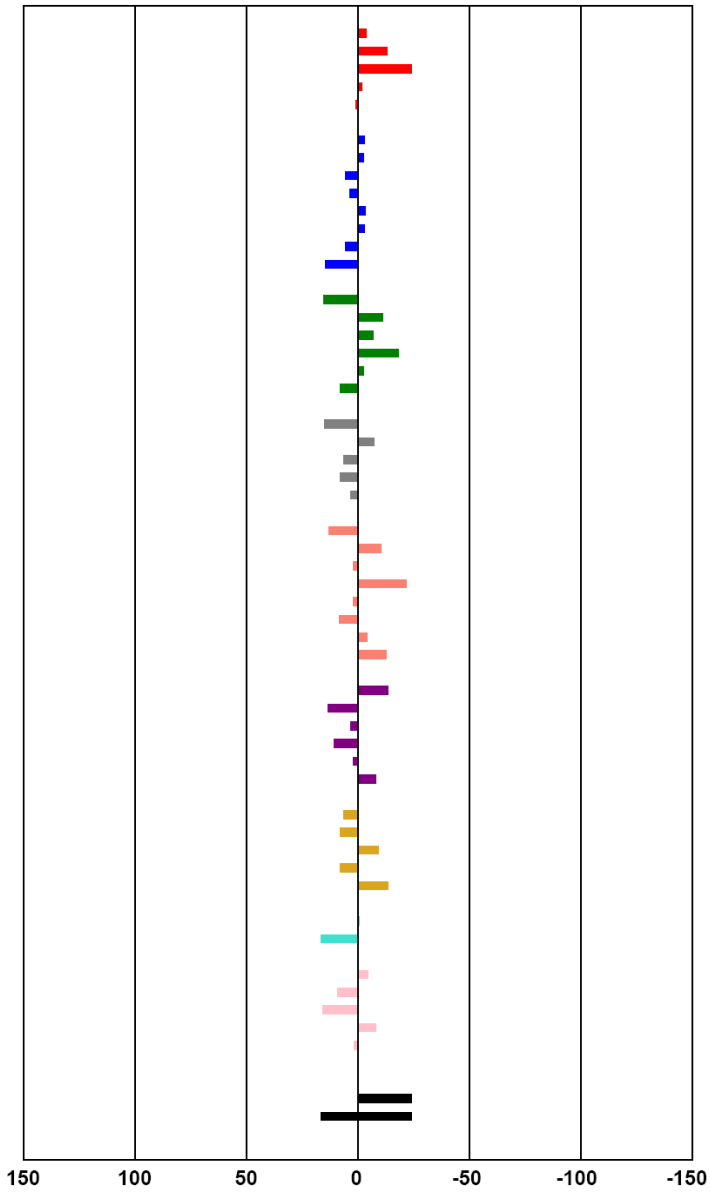
Mean Growth Percent - Growth Percent



Developmental Therapeutics Program	NSC: D-756589 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 14, 2011
One Dose Mean Graph	Experiment ID: 1103OS19		Report Date: Jul 12, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	86.51
HL-60(TB)	77.22
K-562	66.35
MOLT-4	88.51
RPMI-8226	91.04
Non-Small Cell Lung Cancer	
A549/ATCC	87.52
EKVX	88.06
HOP-62	95.60
HOP-92	93.68
NCI-H226	87.10
NCI-H23	87.53
NCI-H322M	95.54
NCI-H460	104.54
Colon Cancer	
COLO 205	105.54
HCT-116	79.11
HCT-15	83.51
HT29	72.19
KM12	87.99
SW-620	97.96
CNS Cancer	
SF-268	104.86
SF-295	83.14
SF-539	96.32
SNB-19	97.98
U251	93.44
Melanoma	
LOX IMVI	102.94
MALME-3M	79.97
M14	92.20
MDA-MB-435	68.85
SK-MEL-28	92.19
SK-MEL-5	98.44
UACC-257	86.43
UACC-62	77.65
Ovarian Cancer	
IGROV1	77.08
OVCAR-3	103.65
OVCAR-4	93.45
OVCAR-5	100.83
OVCAR-8	92.15
NCI/ADR-RES	82.36
Renal Cancer	
A498	96.33
ACHN	98.06
CAKI-1	81.05
SN12C	97.88
UO-31	76.77
Prostate Cancer	
PC-3	89.66
DU-145	106.43
Breast Cancer	
MCF7	86.10
MDA-MB-231/ATCC	99.24
HS 578T	106.01
T-47D	82.29
MDA-MB-468	91.64
Mean	90.22
Delta	23.87
Range	40.08

Mean Growth Percent - Growth Percent



8.4.3. NCI 60 Cell Line Screen - Five-Dose Data

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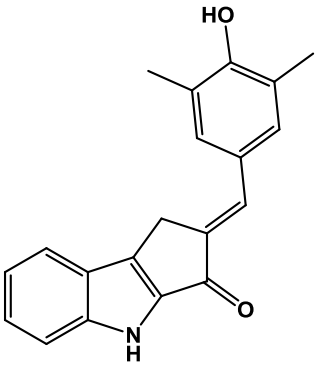

National Cancer Institute Developmental Therapeutics Program
Mean Graphs

NSC : D - 756592/1 | Units :Molar | SSPL :0Y8X | EXP. ID :1105NS51

Report Date :July 12, 2011 | Test Date :May 16, 2011

Panel/Cell Line	Log_{10}GI50	GI50	Log_{10}TGI	TGI	Log_{10}LC50	LC50
Leukemia						
CCRF-CEM	-5.61		> -4.00		> -4.00	
K-562	-6.48		-4.17		> -4.00	
MOLT-4	-5.05		> -4.00		> -4.00	
RPMI-8226	-6.09		> -4.00		> -4.00	
SR	-6.35		> -4.00		> -4.00	
Non-Small Cell Lung Cancer						
EKVX	-5.66		-4.49		> -4.00	
HOP-62	-6.16		-4.50		> -4.00	
HOP-92	-4.84		-4.10		> -4.00	
NCI-H226	-4.86		-4.05		> -4.00	
NCI-H23	-5.75		-4.26		> -4.00	
NCI-H322M	-5.53		> -4.00		> -4.00	
NCI-H460	-6.34		-4.84		-4.06	
NCI-H522	-6.96		-6.12		> -4.00	
Colon Cancer						
COLO 205	-4.75		-4.29		> -4.00	
HCC-2998	-4.96		-4.01		> -4.00	
HCT-116	-6.33		-4.81		-4.16	
HCT-15	-6.18		> -4.00		> -4.00	
HT29	-4.79		-4.39		> -4.00	
KM12	-6.35		-4.74		> -4.00	
SW-620	-6.35		> -4.00		> -4.00	
CNS Cancer						
SF-268	-6.12		> -4.00		> -4.00	
SF-295	-5.62		-4.94		-4.06	
SF-539	-6.20		-4.99		> -4.00	
SNB-19	-6.10		> -4.00		> -4.00	
SNB-75	-5.51		-4.86		> -4.00	
U251	-6.36		-4.74		-4.18	
Melanoma						
LOX IMVI	-6.18		> -4.00		> -4.00	
MALME-3M	-6.50		> -4.00		> -4.00	
M14	-6.38		-4.35		> -4.00	
MDA-MB-435	-6.84		-6.31		-5.36	
SK-MEL-2	-6.47				> -4.00	
SK-MEL-28	-6.13		-4.53		-4.01	
SK-MEL-5	-6.64		-4.86		-4.21	
UACC-257	-4.81		> -4.00		> -4.00	
UACC-62	-5.25		-4.24		> -4.00	
Ovarian Cancer						
IGROV1	-5.58		-4.30		> -4.00	
OVCAR-3	-6.23		-5.58		-4.85	
OVCAR-4	-5.19		> -4.00		> -4.00	
OVCAR-5	-4.97		> -4.00		> -4.00	
OVCAR-8	-6.19		-4.49		> -4.00	
NCI/ADR-RES	-6.39		-4.31		> -4.00	
SK-OV-3	-6.34		-5.01		-4.29	
Renal Cancer						
786-0	-5.88		> -4.00		> -4.00	
A498	-5.18		-4.41		> -4.00	
ACHN			> -4.00		> -4.00	
CAKI-1	-6.18		-4.18		> -4.00	
RXF 393	-6.23		-5.16		> -4.00	
SN12C	-6.13		> -4.00		> -4.00	
UO-31	-6.62		-4.05		> -4.00	
Prostate Cancer						
PC-3	-5.18		> -4.00		> -4.00	
DU-145	-6.04		-4.66		> -4.00	
Breast Cancer						
MCF7	-6.22		> -4.00		> -4.00	
MDA-MB-231/ATCC	-5.22		> -4.00		> -4.00	
HS 578T	-6.26		-4.17		> -4.00	
BT-549			-4.20		> -4.00	
T-47D	-4.89		> -4.00		> -4.00	
MDA-MB-468	-5.59		-5.05		> -4.00	
MID	-5.89		-4.4		-4.06	
Delta	1.07		1.91		1.3	
Range	2.21		2.31		1.36	

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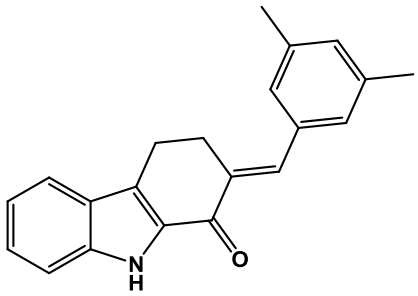

National Cancer Institute Developmental Therapeutics Program Mean Graphs	NSC : D - 756584/1	Units :Molar	SSPL :0Y8X	EXP. ID :1105NS51
	Report Date :July 12, 2011		Test Date :May 16, 2011	

Panel/Cell Line	Log_{10}GI50	GI50	Log_{10}TGI	TGI	Log_{10}LC50	LC50
Leukemia						
CCRF-CEM	-5.69		> -4.00		> -4.00	
K-562	-6.45		> -4.00		> -4.00	
MOLT-4	-5.68		> -4.00		> -4.00	
RPMI-8226	-5.54		> -4.00		> -4.00	
SR	-6.34		-4.74		> -4.00	
Non-Small Cell Lung Cancer						
EKVX	-5.66		> -4.00		> -4.00	
HOP-62	-5.61		-4.19		> -4.00	
HOP-92	-5.10		> -4.00		> -4.00	
NCI-H226	-4.96		> -4.00		> -4.00	
NCI-H23	-5.64		> -4.00		> -4.00	
NCI-H322M	-5.76		> -4.00		> -4.00	
NCI-H460	-6.25		> -4.00		> -4.00	
NCI-H522	-6.46		-5.57		> -4.00	
Colon Cancer						
COLO 205	-6.07		-4.77		> -4.00	
HCC-2998	-5.40		> -4.00		> -4.00	
HCT-116	-6.33		> -4.00		> -4.00	
HCT-15	-6.12		> -4.00		> -4.00	
HT29	-6.51		-4.86		> -4.00	
KM12	-6.24		> -4.00		> -4.00	
SW-620	-6.30		> -4.00		> -4.00	
CNS Cancer						
SF-268	-5.51		> -4.00		> -4.00	
SF-295	-6.08		-4.93		> -4.00	
SF-539	-5.61		-4.33		> -4.00	
SNB-19	-5.49		> -4.00		> -4.00	
SNB-75	-5.77		-5.06		> -4.00	
U251	-6.19		-4.14		> -4.00	
Melanoma						
LOX IMVI	-5.63		> -4.00		> -4.00	
MALME-3M	-6.10		-4.47		> -4.00	
M14	-6.26		-4.89		> -4.00	
MDA-MB-435	-6.81		-6.35		-5.70	
SK-MEL-2	-6.34		-5.47		> -4.00	
SK-MEL-28	-5.85		-4.04		> -4.00	
SK-MEL-5	-6.36		-5.22		-4.35	
UACC-257	-5.52		-4.02		> -4.00	
UACC-62	-6.09		-4.10		> -4.00	
Ovarian Cancer						
IGROV1	-5.88		> -4.00		> -4.00	
OVCAR-3	-6.44		-5.85		-4.91	
OVCAR-4	-5.60		> -4.00		> -4.00	
OVCAR-5	-5.05		> -4.00		> -4.00	
OVCAR-8	-5.60		> -4.00		> -4.00	
NCI/ADR-RES	-6.33		-5.02		> -4.00	
SK-OV-3	-5.96		-4.96		> -4.00	
Renal Cancer						
786-0	-5.51		> -4.00		> -4.00	
A498	-5.96		-4.73		> -4.00	
ACHN	-5.13		> -4.00		> -4.00	
CAKI-1	-6.04		> -4.00		> -4.00	
RXF 393	-5.75		-5.07		> -4.00	
SN12C	-5.44		> -4.00		> -4.00	
UO-31	-5.75		> -4.00		> -4.00	
Prostate Cancer						
PC-3	-5.44		> -4.00		> -4.00	
DU-145	-5.63		-4.46		> -4.00	
Breast Cancer						
MCF7	-5.41		-4.61		> -4.00	
MDA-MB-231/ATCC	-4.84		> -4.00		> -4.00	
HS 578T	-5.74		-4.68		> -4.00	
BT-549	-5.29		> -4.00		> -4.00	
T-47D	-5.74		> -4.00		> -4.00	
MDA-MB-468	-6.45		-5.47		-4.11	
MID	-5.85		-4.39		-4.05	
Delta	0.96		1.96		1.65	
Range	1.97		2.35		1.7	

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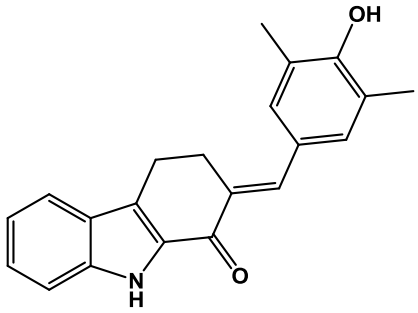


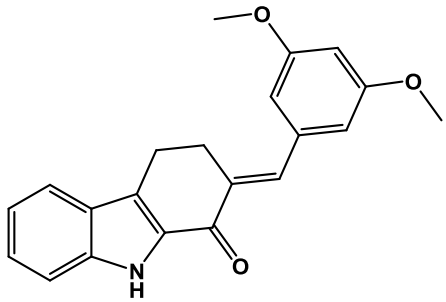
National Cancer Institute Developmental Therapeutics Program
Mean Graphs

NSC : D - 756586/1 | Units :Molar | SSPL :0Y8X | EXP. ID :1105NS51

Report Date :July 12, 2011 | Test Date :May 16, 2011

Panel/Cell Line	Log_{10}GI50	Log_{10}TGI	Log_{10}LC50
Leukemia			
CCRF-CEM	-6.46	-4.67	> -4.00
K-562	-6.49	-4.79	> -4.00
MOLT-4	-6.17	-4.88	> -4.00
RPMI-8226	-6.33	> -4.00	> -4.00
SR	-6.53	-4.75	> -4.00
Non-Small Cell Lung Cancer			
EKVX	-5.45	-4.80	-4.37
HOP-62	-6.21	-4.75	-4.32
HOP-92	-5.96	-4.82	-4.31
NCI-H226	-5.35	-4.73	-4.36
NCI-H23	-6.19	-4.85	-4.12
NCI-H322M	-6.31	-4.79	-4.39
NCI-H460	-6.39	-5.08	-4.31
NCI-H522	-7.06	-6.22	-4.89
Colon Cancer			
COLO 205	-4.81	-4.53	-4.25
HCC-2998	-5.53	-4.96	-4.44
HCT-116	-6.34	-4.96	-4.44
HCT-15	-6.31	-4.84	-4.19
HT29	-5.27	-4.70	-4.20
KM12	-6.42	-5.60	-4.66
SW-620	-6.37	-4.84	-4.37
CNS Cancer			
SF-268	-6.12	-4.84	-4.37
SF-295	-5.80	-5.09	-4.51
SF-539	-6.46	-4.93	-4.22
SNB-19	-6.18	-4.73	-4.35
SNB-75	-6.48	-5.10	-4.48
U251	-6.39	-4.94	-4.38
Melanoma			
LOX IMVI	-6.07	-4.87	-4.25
MALME-3M	-6.43	-4.72	-4.32
M14	-6.49	-4.94	-4.33
MDA-MB-435	-6.99	-6.49	-4.97
SK-MEL-2	-6.54		-4.40
SK-MEL-28	-6.12	-4.77	-4.37
SK-MEL-5	-6.59	-6.06	-4.78
UACC-257	-6.44	-4.79	-4.38
UACC-62	-6.35	-4.82	-4.38
Ovarian Cancer			
IGROV1	-6.19	-4.80	-4.33
OVCAR-3	-6.47	-6.08	-5.24
OVCAR-4	-6.12	-4.86	-4.40
OVCAR-5	-5.57	-4.75	-4.15
OVCAR-8	-6.29	-4.98	-4.29
NCI/ADR-RES	-6.53	-4.96	> -4.00
SK-OV-3	-6.33	-4.94	-4.47
Renal Cancer			
786-0	-6.25	-4.86	-4.36
A498	-5.66	-4.98	-4.48
ACHN	-6.08	-4.82	-4.37
CAKI-1	-6.29	-4.82	-4.38
RXF 393	-6.50	-5.01	-4.45
SN12C	-6.12	-4.73	-4.20
UO-31	-6.36	-4.89	-4.44
Prostate Cancer			
PC-3	-5.81	-4.56	> -4.00
DU-145	-6.34	-4.97	-4.46
Breast Cancer			
MCF7	-6.45	-4.95	-4.43
MDA-MB-231/ATCC	-5.83	-4.86	-4.38
HS 578T	-6.41	-4.23	> -4.00
BT-549	-6.30	-4.78	-4.36
T-47D	-6.08	-4.67	-4.07
MDA-MB-468	-6.51	-5.33	-4.49
MID	-6.21	-4.94	-4.35
Delta	0.85	1.55	0.89
Range	2.25	2.49	1.24

188



National Cancer Institute Developmental Therapeutics Program
Mean Graphs

NSC : D - 756585/1 | Units :Molar | SSPL :0Y8X | EXP. ID :1105NS51

Report Date :July 12, 2011 | Test Date :May 16, 2011

Panel/Cell Line	$Log_{10}GI50$	GI50	$Log_{10}TGI$	TGI	$Log_{10}LC50$	LC50
Leukemia						
CCRF-CEM	-5.30		> -4.00		> -4.00	
K-562	-5.42		> -4.00		> -4.00	
MOLT-4	-5.32		-4.17		> -4.00	
RPMI-8226	-5.05		> -4.00		> -4.00	
SR	-5.36		> -4.00		> -4.00	
Non-Small Cell Lung Cancer						
EKVX	-5.41		> -4.00		> -4.00	
HOP-62	-4.87		> -4.00		> -4.00	
HOP-92	-4.98		-4.26		> -4.00	
NCI-H226	-4.81		> -4.00		> -4.00	
NCI-H23	-5.05		> -4.00		> -4.00	
NCI-H322M	-4.88		> -4.00		> -4.00	
NCI-H460	-5.31		-4.67		> -4.00	
NCI-H522	-5.60		-5.11		> -4.00	
Colon Cancer						
COLO 205	-4.79		-4.22		> -4.00	
HCC-2998	-4.78		> -4.00		> -4.00	
HCT-116	-5.40		-4.50		> -4.00	
HCT-15	-5.22		> -4.00		> -4.00	
HT29	-5.47		-4.83		> -4.00	
KM12	-5.28		-4.46		> -4.00	
SW-620	-5.29		> -4.00		> -4.00	
CNS Cancer						
SF-268	-4.93		-4.00		> -4.00	
SF-295	-5.41		> -4.00		> -4.00	
SF-539	-4.97		-4.32		> -4.00	
SNB-19	-4.90		> -4.00		> -4.00	
SNB-75	-5.03		-4.42		> -4.00	
U251	-5.35		> -4.00		> -4.00	
Melanoma						
LOX IMVI	-5.10		> -4.00		> -4.00	
MALME-3M	-5.51		-4.46		> -4.00	
M14	-5.44		> -4.00		> -4.00	
MDA-MB-435	-5.82		-5.48			
SK-MEL-2	-5.60		-5.04		> -4.00	
SK-MEL-28	-4.86		> -4.00		> -4.00	
SK-MEL-5	-5.51		-4.91		-4.44	
UACC-257	-4.88		> -4.00		> -4.00	
UACC-62	-5.35		> -4.00		> -4.00	
Ovarian Cancer						
IGROV1	-5.23		> -4.00		> -4.00	
OVCAR-3	-5.40		-4.89		> -4.00	
OVCAR-4	-5.14		> -4.00		> -4.00	
OVCAR-5	-4.75		> -4.00		> -4.00	
OVCAR-8	-5.15		> -4.00		> -4.00	
NCI/ADR-RES	-5.45		> -4.00		> -4.00	
SK-OV-3	-5.10		-4.30		> -4.00	
Renal Cancer						
786-0	-4.93		> -4.00		> -4.00	
A498	-5.12		-4.23		> -4.00	
ACHN	-4.68		> -4.00		> -4.00	
CAKI-1	-5.23		> -4.00		> -4.00	
RXF 393	-5.06		-4.47		> -4.00	
SN12C	-4.96		-4.08		> -4.00	
UO-31	-5.17		> -4.00		> -4.00	
Prostate Cancer						
PC-3	-4.81		> -4.00		> -4.00	
DU-145	-4.84		-4.27		> -4.00	
Breast Cancer						
MCF7	-5.47		-4.32		> -4.00	
MDA-MB-231/ATCC	-5.13		> -4.00		> -4.00	
HS 578T	-5.15		-4.26		> -4.00	
BT-549	-5.27		-4.17		> -4.00	
T-47D	-5.10		> -4.00		> -4.00	
MDA-MB-468	-5.70		-5.11		> -4.00	
MID	-5.17		-4.23		-4.01	
Delta	0.65		1.25		0.43	
Range	1.14		1.48		0.44	

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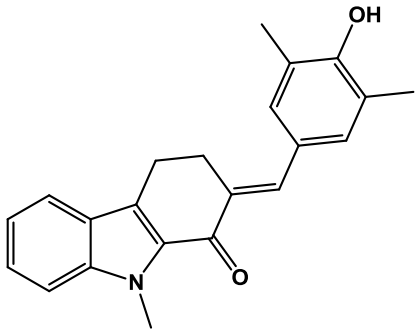

National Cancer Institute Developmental Therapeutics Program Mean Graphs	NSC : D - 756590/1	Units :Molar	SSPL :0Y8X	EXP. ID :1105NS51
	Report Date :July 12, 2011		Test Date :May 16, 2011	

Panel/Cell Line	$Log_{10}GI50$	GI50	$Log_{10}TGI$	TGI	$Log_{10}LC50$	LC50
Leukemia						
CCRF-CEM	-6.04		> -4.00		> -4.00	
K-562	-6.48		-4.89		> -4.00	
MOLT-4	-6.16		> -4.00		> -4.00	
RPMI-8226	-5.97		> -4.00		> -4.00	
SR	-6.44		-4.67		> -4.00	
Non-Small Cell Lung Cancer						
EKVX	-5.37		-4.50		> -4.00	
HOP-62	-6.14		-4.81		-4.33	
HOP-92	-4.98		-4.46		> -4.00	
NCI-H226	-5.40		-4.66		-4.04	
NCI-H23	-5.60		-4.31		> -4.00	
NCI-H322M	-5.75		-4.93		-4.14	
NCI-H460	-6.38		-4.89		> -4.00	
NCI-H522	-6.88		-6.16		> -4.00	
Colon Cancer						
COLO 205	-4.70		> -4.00		> -4.00	
HCC-2998	-5.38		> -4.00		> -4.00	
HCT-116	-6.43		-4.63		> -4.00	
HCT-15	-6.22		-4.01		> -4.00	
HT29	-5.31		> -4.00		> -4.00	
KM12	-6.34		-4.65		> -4.00	
SW-620	-6.33		> -4.00		> -4.00	
CNS Cancer						
SF-268	-6.17		-4.72		-4.05	
SF-295	-5.96		-5.26		-4.43	
SF-539	-6.05		-4.94		-4.02	
SNB-19	-6.15		-4.67		-4.03	
SNB-75	-6.27		-5.38		-4.63	
U251	-6.35		-4.92		-4.19	
Melanoma						
LOX IMVI	-6.10		> -4.00		> -4.00	
MALME-3M	-6.49		-5.15		> -4.00	
M14	-6.43		-4.70		> -4.00	
MDA-MB-435	-6.83		-6.38		-4.94	
SK-MEL-2	-6.48		-5.55		-4.32	
SK-MEL-28	-6.25		-4.80		-4.09	
SK-MEL-5	-6.55		-5.98		-5.12	
UACC-257	-5.78		-4.76		-4.09	
UACC-62	-6.32		-4.78		-4.05	
Ovarian Cancer						
IGROV1	-6.04		-4.66		> -4.00	
OVCAR-3	-6.47		-5.95		-4.72	
OVCAR-4	-5.55		-4.65		> -4.00	
OVCAR-5	-5.27		-4.37		> -4.00	
OVCAR-8	-6.18		-4.89		-4.18	
NCI/ADR-RES	-6.43		-4.92		> -4.00	
SK-OV-3	-6.06		-4.93		-4.38	
Renal Cancer						
786-0	-5.80		-4.72		-4.03	
A498	-5.36		-4.80		-4.23	
ACHN	-6.08		-4.66		-4.03	
CAKI-1	-6.34		-4.78		> -4.00	
RXF 393	-6.12		-5.34		-4.50	
SN12C	-6.00		-4.42		> -4.00	
UO-31	-6.31		-4.57		> -4.00	
Prostate Cancer						
PC-3	-5.39		-4.51		> -4.00	
DU-145	-5.98		-5.22		-4.38	
Breast Cancer						
MCF7	-6.44		-4.90		-4.05	
MDA-MB-231/ATCC	-5.85		-4.68		> -4.00	
HS 578T	-6.08		-4.64		> -4.00	
BT-549	-5.94		-4.74		-4.22	
T-47D	-5.63		-4.77		> -4.00	
MDA-MB-468	-6.55		-5.84		-4.68	
MID	-6.04		-4.78		-4.14	
Delta	0.84		1.6		0.98	
Range	2.18		2.38		1.12	

8.4.4. Repeat of Five-Dose Data

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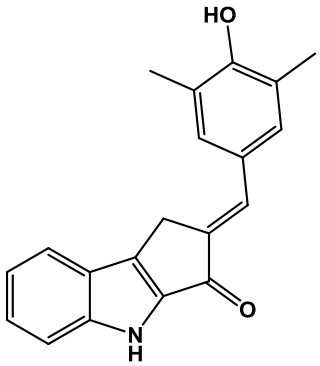

National Cancer Institute Developmental Therapeutics Program
Mean Graphs

NSC : D - 756592/1 | Units :Molar | SSPL :0Y8X | EXP. ID :1107RS89

Report Date :September 27, 2011 | Test Date :July 25, 2011

Panel/Cell Line	$Log_{10}GI50$	GI50	$Log_{10}TGI$	TGI	$Log_{10}LC50$	LC50
Leukemia						
CCRF-CEM	-5.72		> -4.00		> -4.00	
HL-60(TB)	-6.47		-6.00		> -4.00	
MOLT-4	-5.52		> -4.00		> -4.00	
RPMI-8226	-6.28		> -4.00		> -4.00	
SR	-6.48		-4.42		> -4.00	
Non-Small Cell Lung Cancer						
A549/ATCC	-5.80		-4.70		-4.09	
EKVX	-5.52		> -4.00		> -4.00	
HOP-62	-6.21		-4.61		-4.04	
HOP-92	-6.27		-4.24		> -4.00	
NCI-H226	-5.26		> -4.00		> -4.00	
NCI-H322M	-4.75		> -4.00		> -4.00	
NCI-H460	-6.41		-4.93		> -4.00	
NCI-H522	-7.10		-6.20		> -4.00	
Colon Cancer						
COLO 205	-4.67		-4.25		> -4.00	
HCC-2998	-5.40		-4.03		> -4.00	
HCT-116	-6.27		-4.82		-4.23	
HCT-15	-6.33		> -4.00		> -4.00	
HT29	-4.81		-4.40		> -4.00	
KM12	-6.32		-4.51		> -4.00	
SW-620	-6.43		> -4.00		> -4.00	
CNS Cancer						
SF-268	-6.02		> -4.00		> -4.00	
SF-295	-5.60		-4.82		> -4.00	
SF-539	-6.34		-5.19		> -4.00	
SNB-19	-6.20		> -4.00		> -4.00	
SNB-75	-6.56		-4.53		> -4.00	
U251	-5.75		-4.63		> -4.00	
Melanoma						
LOX IMVI	-6.15		> -4.00		> -4.00	
MALME-3M			-4.24		> -4.00	
M14	-6.38		-4.54		> -4.00	
MDA-MB-435	-6.75		-6.31		-4.64	
SK-MEL-2	-6.35		-4.45		> -4.00	
SK-MEL-28			-4.45		> -4.00	
SK-MEL-5	-6.31		-4.70		-4.03	
UACC-257	-4.44		> -4.00		> -4.00	
UACC-62	-5.45		-4.53		> -4.00	
Ovarian Cancer						
IGROV1	-5.35		-4.17		> -4.00	
OVCAR-3	-6.38		-5.58		-4.32	
OVCAR-4	-4.85		-4.06		> -4.00	
OVCAR-5	-4.94		-4.10		> -4.00	
OVCAR-8	-5.87		-4.25		> -4.00	
NCI/ADR-RES	-6.54		-5.70		> -4.00	
SK-OV-3	-6.00		-4.81		-4.04	
Renal Cancer						
786-0	-4.98		-4.13		> -4.00	
A498	-5.51		-4.63		> -4.00	
ACHN			> -4.00		> -4.00	
CAKI-1	-5.67		> -4.00		> -4.00	
RXF 393	-5.64		-4.66		> -4.00	
SN12C	-6.30		-4.54		> -4.00	
TK-10	-5.20		-4.40		> -4.00	
UO-31	-6.41		-4.39		> -4.00	
Prostate Cancer						
PC-3	-6.26		> -4.00		> -4.00	
DU-145	-5.50		> -4.00		> -4.00	
Breast Cancer						
MCF7	-6.10		> -4.00		> -4.00	
MDA-MB-231/ATCC	-6.43		-4.87		> -4.00	
HS 578T	-6.26		> -4.00		> -4.00	
BT-549			-4.18		> -4.00	
T-47D	-4.91		> -4.00		> -4.00	
MDA-MB-468	-5.64		-5.07		> -4.00	
MID	-5.87		-4.46		-4.02	
Delta	1.23		1.85		0.62	
Range	2.66		2.31		0.64	

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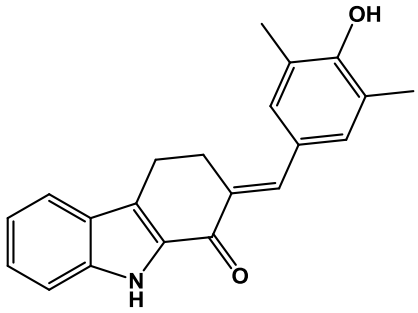

National Cancer Institute Developmental Therapeutics Program Mean Graphs	NSC : D - 756586/1	Units :Molar	SSPL :0Y8X	EXP. ID :1107RS89
	Report Date :September 27, 2011		Test Date :July 25, 2011	

Panel/Cell Line	$Log_{10}GI50$	GI50	$Log_{10}TGI$	TGI	$Log_{10}LC50$	LC50
Leukemia						
CCRF-CEM	-6.42		-4.64		> -4.00	
HL-60(TB)	-6.67		-6.27		-4.01	
MOLT-4	-6.36		-4.89		> -4.00	
RPMI-8226	-6.33		-4.72		> -4.00	
SR	-6.84		-5.73		> -4.00	
Non-Small Cell Lung Cancer						
A549/ATCC	-6.26		-4.92		-4.39	
EKVX	-5.50		-4.85		-4.42	
HOP-62	-6.16		-4.75		-4.17	
HOP-92	-6.33		-4.89		-4.24	
NCI-H226	-5.68		-4.92		-4.46	
NCI-H322M	-6.24		-4.89		-4.44	
NCI-H460	-6.38		-4.98		-4.34	
NCI-H522	-6.79		-6.17		-4.63	
Colon Cancer						
COLO 205	-4.89		-4.58		-4.28	
HCC-2998	-5.84		-5.15		-4.51	
HCT-116	-6.36		-4.97		-4.41	
HCT-15	-6.46		-5.30		-4.47	
HT29	-5.48		-4.90		-4.33	
KM12	-6.41		-4.99		-4.49	
SW-620	-6.41		-4.87		-4.43	
CNS Cancer						
SF-268	-6.15		-4.88		-4.44	
SF-295	-5.66		-5.17		-4.58	
SF-539	-6.53		-5.95		-4.32	
SNB-19	-6.30		-4.86		-4.43	
SNB-75	-6.60		-4.75		> -4.00	
U251	-6.35		-4.86		-4.35	
Melanoma						
LOX IMVI	-6.18		-4.89		-4.39	
MALME-3M	-6.41		-4.77		-4.38	
M14	-6.44		-4.98		-4.43	
MDA-MB-435	-6.85		-6.44			
SK-MEL-2	-6.46		-4.86		-4.33	
SK-MEL-28	-6.15		-4.79		-4.39	
SK-MEL-5	-6.39		-4.92		-4.46	
UACC-257	-6.43		-4.81		-4.40	
UACC-62	-6.41		-5.19		-4.52	
Ovarian Cancer						
IGROV1	-6.08		-4.88		-4.26	
OVCAR-3	-6.50		-6.01		-4.66	
OVCAR-4	-5.71		-4.83		-4.41	
OVCAR-5	-5.70		-4.97		-4.32	
OVCAR-8	-6.27		-4.96		-4.29	
NCI/ADR-RES	-6.48		-5.38		> -4.00	
SK-OV-3	-6.33		-5.15		-4.53	
Renal Cancer						
786-0	-5.92		-4.91		-4.43	
A498	-5.84		-5.23		-4.62	
ACHN	-6.15		-4.92		-4.46	
CAKI-1	-6.15		-4.76		-4.36	
RXF 393	-6.14		-4.95		-4.44	
SN12C	-6.32		-4.96		-4.43	
TK-10	-6.27		-4.94		-4.46	
UO-31	-6.33		-5.28		-4.56	
Prostate Cancer						
PC-3	-6.25		-4.62		> -4.00	
DU-145	-6.21		-4.93		-4.47	
Breast Cancer						
MCF7	-6.46		-4.96		-4.46	
MDA-MB-231/ATCC	-6.20		-5.36		-4.56	
HS 578T	-6.44		-4.35		> -4.00	
BT-549	-6.26		-4.72		-4.33	
T-47D	-6.18		-4.83		-4.16	
MDA-MB-468	-6.58		-6.01		-4.63	
MID	-6.24		-5.06		-4.36	
Delta	0.61		1.38		0.3	
Range	1.96		2.09		0.66	

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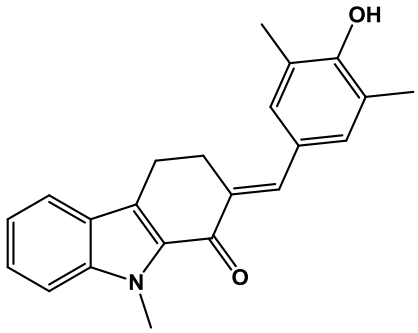

National Cancer Institute Developmental Therapeutics Program Mean Graphs	NSC : D - 756590/1	Units :Molar	SSPL :0Y8X	EXP. ID :1107RS89
	Report Date :September 27, 2011		Test Date :July 25, 2011	

Panel/Cell Line	$Log_{10}GI50$	GI50	$Log_{10}TGI$	TGI	$Log_{10}LC50$	LC50
Leukemia						
CCRF-CEM	-6.25		> -4.00		> -4.00	
HL-60(TB)	-6.46		-5.21		> -4.00	
MOLT-4	-6.04		> -4.00		> -4.00	
RPMI-8226	-6.34		-4.76		> -4.00	
SR	-6.49		-4.96		> -4.00	
Non-Small Cell Lung Cancer						
A549/ATCC	-6.13		-4.69		> -4.00	
EKVX	-5.27		> -4.00		> -4.00	
HOP-62	-6.14		-4.72		-4.26	
HOP-92	-5.58		-4.62		-4.14	
NCI-H226	-5.32		-4.54		> -4.00	
NCI-H322M	-5.43		-4.67		-4.03	
NCI-H460	-6.42		-4.87		> -4.00	
NCI-H522	-6.86		-6.18		> -4.00	
Colon Cancer						
COLO 205	-4.59		> -4.00		> -4.00	
HCC-2998	-5.52		> -4.00		> -4.00	
HCT-116	-6.38		-4.72		> -4.00	
HCT-15	-6.38		-4.64		> -4.00	
HT29	-5.47		> -4.00		> -4.00	
KM12	-6.35		-4.58		> -4.00	
SW-620	-6.41		> -4.00		> -4.00	
CNS Cancer						
SF-268	-6.11		-4.67		-4.04	
SF-295	-5.73		-5.06		-4.41	
SF-539	-6.28		-5.25		-4.44	
SNB-19	-6.26		-4.70		-4.15	
SNB-75	-5.53		-4.96		-4.20	
U251	-6.20		-4.78		-4.09	
Melanoma						
LOX IMVI	-6.22		> -4.00		> -4.00	
MALME-3M	-6.24		-4.94		> -4.00	
M14	-6.39		-4.71		> -4.00	
MDA-MB-435	-6.74		-6.32		-4.37	
SK-MEL-2	-6.47		-4.92		-4.06	
SK-MEL-28	-6.15		-4.50		> -4.00	
SK-MEL-5	-6.27		-4.84		-4.28	
UACC-257	-5.00		-4.39		> -4.00	
UACC-62	-6.48		-4.90		-4.39	
Ovarian Cancer						
IGROV1	-5.65		-4.53		> -4.00	
OVCAR-3	-6.44		-5.79		-4.49	
OVCAR-4	-5.31		-4.60		-4.11	
OVCAR-5	-5.32		-4.52		> -4.00	
OVCAR-8	-5.93		-4.54		> -4.00	
NCI/ADR-RES	-6.52		-4.95		> -4.00	
SK-OV-3	-5.66		-4.81		-4.33	
Renal Cancer						
786-0	-5.31		-4.62		-4.10	
A498	-5.61		-4.95		-4.21	
ACHN	-6.08		-4.59		> -4.00	
CAKI-1	-5.91		> -4.00		> -4.00	
RXF 393	-5.85		-4.81		-4.21	
SN12C	-6.35		-4.83		-4.10	
TK-10	-5.96		-4.79		-4.29	
UO-31	-6.02		-4.59		> -4.00	
Prostate Cancer						
PC-3	-6.07		-4.67		> -4.00	
DU-145	-5.58		-4.83		-4.11	
Breast Cancer						
MCF7	-6.41		-4.84		-4.17	
MDA-MB-231/ATCC	-6.16		-5.01		-4.22	
HS 578T	-6.21		-4.28		> -4.00	
BT-549	-5.34		-4.63		-4.19	
T-47D	-5.76		-4.62		> -4.00	
MDA-MB-468	-6.48		-5.35		-4.28	
MID	-6.01		-4.71		-4.1	
Delta	0.85		1.61		0.39	
Range	2.27		2.32		0.49	

8.4.5. COMPARE Data of Compound 177

GI_{50}

Rank	Correlation	namecode	Seed Vector ident For Display	Seed Vector descriptor For Display	Target Vector ident For Display	Target Vector descriptor For Display	Count Common Cell Lines	Seed Standard Deviation	Target Standard Deviation
1	0.383	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S153858 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0	maytansine	57	0.567	0.666
2	0.371	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S104801 Endpt:GI50 ExpId:AVGDATA hiConc:-3.0	cytembena	53	0.563	0.256
3	0.35	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S95441 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0	methyl-CCNU	48	0.575	0.493
4	0.344	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S332598 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0	rhizoxin	56	0.557	0.352
5	0.339	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S49842 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0	vinblastine sulfate	55	0.569	0.563
6	0.332	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S153858 Endpt:GI50 ExpId:AVGDATA hiConc:-7.0	maytansine	56	0.568	0.729
7	0.324	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S45388 Endpt:GI50 ExpId:AVGDATA hiConc:-3.0	DTIC	54	0.564	0.55
8	0.323	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S141537 Endpt:GI50 ExpId:AVGDATA hiConc:-7.0	anguidine	55	0.56	0.295
9	0.32	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S163501 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0	AT-125 (acivicin)	52	0.544	0.44
10	0.318	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S332598 Endpt:GI50 ExpId:AVGDATA hiConc:-9.0	rhizoxin	55	0.549	0.631
11	0.301	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S119875 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0	cisplatin	58	0.564	0.324
12	0.291	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S182986 Endpt:GI50 ExpId:AVGDATA hiConc:-4.6	AZQ	53	0.565	0.507
13	0.291	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S322921 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0	pibenzimol hydrochloride	56	0.571	0.438

Rank	Correlation	namecode	Seed Vector ident For Display	Seed Vector descriptor For Display	Target Vector ident For Display	Target Vector descriptor For Display	Count Common Cell Lines	Seed Standard Deviation	Target Standard Deviation
14	0.289	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S224131 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0	PALA	54	0.58	0.065
15	0.289	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S224131 Endpt:GI50 ExpId:AVGDATA hiConc:-2.0	PALA	56	0.559	0.73
16	0.289	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S283162 Endpt:GI50 ExpId:AVGDATA hiConc:-2.9	trimethyltrimethylolmelamine	55	0.56	0.229
17	0.286	PUBLIC	NSC:S756592 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0		NSC:S125973 Endpt:GI50 ExpId:AVGDATA hiConc:-6.0	paclitaxel (Taxol)	58	0.564	0.517

TGI

Rank	Correlation	namecode	Seed Vector ident For Display	Seed Vector descriptor For Display	Target Vector ident For Display	Target Vector descriptor For Display	Count Common Cell Lines	Seed Standard Deviation	Target Standard Deviation
1	0.467	PUBLIC	NSC:S756592 Endpt:TGI ExpId:AVGDATA hiConc:-4.0		NSC:S218321 Endpt:GI50 ExpId:AVGDATA hiConc:-3.2	2'-deoxycoformycin	41	0.52	0.066
2	0.441	PUBLIC	NSC:S756592 Endpt:TGI ExpId:AVGDATA hiConc:-4.0		NSC:S224131 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0	PALA	56	0.538	0.064
3	0.374	PUBLIC	NSC:S756592 Endpt:TGI ExpId:AVGDATA hiConc:-4.0		NSC:S320846 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0	batracylin	44	0.507	0.271
4	0.353	PUBLIC	NSC:S756592 Endpt:TGI ExpId:AVGDATA hiConc:-4.0		NSC:S141537 Endpt:GI50 ExpId:AVGDATA hiConc:-9.0	anguidine	47	0.508	0.061
5	0.332	PUBLIC	NSC:S756592 Endpt:TGI ExpId:AVGDATA hiConc:-4.0		NSC:S125973 Endpt:GI50 ExpId:AVGDATA hiConc:-6.0	paclitaxel (Taxol)	60	0.532	0.533
6	0.305	PUBLIC	NSC:S756592 Endpt:TGI ExpId:AVGDATA hiConc:-4.0		NSC:S320846 Endpt:GI50 ExpId:AVGDATA hiConc:-3.9	batracylin	58	0.474	0.343
7	0.295	PUBLIC	NSC:S756592 Endpt:TGI ExpId:AVGDATA hiConc:-4.0		NSC:S241240 Endpt:GI50 ExpId:AVGDATA hiConc:-3.6	CBDCA (carboplatin)	60	0.532	0.24
8	0.29	PUBLIC	NSC:S756592 Endpt:TGI ExpId:AVGDATA hiConc:-4.0		NSC:S326231 Endpt:GI50 ExpId:AVGDATA hiConc:-2.3	L-buthionine sulfoximine	57	0.477	0.307
9	0.286	PUBLIC	NSC:S756592 Endpt:TGI ExpId:AVGDATA hiConc:-4.0		NSC:S153858 Endpt:GI50 ExpId:AVGDATA hiConc:-7.0	maytansine	58	0.535	0.72
10	0.269	PUBLIC	NSC:S756592 Endpt:TGI ExpId:AVGDATA hiConc:-4.0		NSC:S83265 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0	S-trityl-L-cysteine	47	0.496	0.596
11	0.268	PUBLIC	NSC:S756592 Endpt:TGI ExpId:AVGDATA hiConc:-4.0		NSC:S49842 Endpt:GI50 ExpId:AVGDATA hiConc:-5.6	vinblastine sulfate	60	0.532	0.843
12	0.261	PUBLIC	NSC:S756592 Endpt:TGI ExpId:AVGDATA hiConc:-4.0		NSC:S15200 Endpt:GI50 ExpId:AVGDATA hiConc:-2.0	gallium nitrate	57	0.536	0.516
13	0.256	PUBLIC	NSC:S756592 Endpt:TGI ExpId:AVGDATA hiConc:-4.0		NSC:S153858 Endpt:GI50 ExpId:AVGDATA hiConc:-8.6	maytansine	41	0.533	1.592

LC_{50}

Rank	Correlation	namecode	Seed Vector ident For Display	Seed Vector descriptor For Display	Target Vector ident For Display	Target Vector descriptor For Display	Count Common Cell Lines	Seed Standard Deviation	Target Standard Deviation
1	0.592	PUBLIC	NSC:S756592 Endpt:LC50 ExpId:AVGDATA hiConc:-4.0		NSC:S224131 Endpt:GI50 ExpId:AVGDATA hiConc:-4.0	PALA	56	0.155	0.064
2	0.434	PUBLIC	NSC:S756592 Endpt:LC50 ExpId:AVGDATA hiConc:-4.0		NSC:S153858 Endpt:GI50 ExpId:AVGDATA hiConc:-7.0	maytansine	58	0.152	0.72
3	0.339	PUBLIC	NSC:S756592 Endpt:LC50 ExpId:AVGDATA hiConc:-4.0		NSC:S314055 Endpt:GI50 ExpId:AVGDATA hiConc:-2.9	SR2555 (nitroimidazole)	46	0.093	0.344
4	0.273	PUBLIC	NSC:S756592 Endpt:LC50 ExpId:AVGDATA hiConc:-4.0		NSC:S125973 Endpt:GI50 ExpId:AVGDATA hiConc:-6.0	paclitaxel (Taxol)	60	0.15	0.533

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